METHODS AND COMPOSITIONS FOR DIAGNOSIS, STAGING AND PROGNOSIS OF PROSTATE CANCER

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of priority to U.S. Provisional Application No. 60/487,553 filed 14 July 2003, and incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to novel methods and compositions for the diagnosis, staging, prognosis and treatment of prostate cancer, based on genomic markers for genomic DNA methylation and/or gene expression, including transcriptional silencing, and/or based on protein markers. Particular embodiments provide methods, nucleic acids, nucleic acid arrays and kits useful for detecting, or for detecting and differentiating between or among prostate cell proliferative disorders and/or tumor progression.

STATEMENT REGARDING FEDERALLY FUNDED RESEARCH

This work was, at least in part, supported by National Institutes of Health Grants CA91956 and CA70892, and the United States Government has certain rights in the invention.

BACKGROUND

Currently, tumor stage, Gleason score, and preoperative serum PSA are the only well-recognized predictors of prostate cancer progression. However, these markers cannot reliably identify men that ultimately fail therapy, and give no insight into prostate carcinogenesis, or potential therapeutic targets for prostate cancer.

Prostate cancer initiation and progression are processes involving multiple molecular alterations, including alteration of gene, and gene product expression. Identification of these differentially expressed genes represents a critical step towards a thorough understanding of prostate carcinogenesis and an improved management (*e.g.*, diagnostic and/or prognostic) of prostate cancer patients.

Inactivation of tumor suppression genes is an important event contributing to the development of neoplastic malignancies. In addition to the classical genetic mechanisms involving deletion or activating point mutations, growth regulatory genes can be functionally inactivated or otherwise modulated by *epigenetic* alterations; for example, alterations in the genome other than the

DNA sequence itself, which include genomic hypomethylations, promoter-related hypermethylation (*e.g.*, of CpG dinucleotides, and CpG islands), histone deacetylation and chromatin modifications. Molecular analysis of tumor-derived genetic and epigenetic alterations may have a profound impact on cancer diagnosis and monitoring for tumor recurrence.

Therefore, there is a need in the art to identify differentially expressed genes (*e.g.*, using s) between cancer and corresponding normal tissues to advance the understanding of the molecular basis of malignancy, and to provide diagnostic and/or prognostic markers of malignancy and methods for using these markers, as well as to provide novel therapeutic targets and corresponding methods of treatment.

There is a need in the art to identify and statistically correlate altered gene expression that is characteristic of the specific stage of the cancer to provide compositions and methods that are independent and/or supplementary to the standard histopathological approaches to work-up of precancerous and cancerous lesions of the prostate.

SUMMARY OF THE INVENTION

Genes expression was profiled in benign and untreated human prostate cancer tissues using oligonucleotide s. Six hundred seventy-four (674) genes with distinct (*i.e.*, differential expression relative to benign tissue) expression patterns in metastatic and confined tumors (Gleason score 6 and 9, lymph node invasive and non-invasive) were identified. Validation of expression profiles of seventeen (17) genes by quantitative PCR revealed a strong *inverse* correlation in the expression with progression of prostate cancer for: zinc finger protein (ZNF185), bullous pemphigoid antigen gene (BPAG1), prostate secretory protein (PSP94) (see EXAMPLE I below); and for supervillin (SVIL); proline rich membrane anchor 1 (PRIMA1); TU3A; FLJ14084; KIAA1210; sorbin and SH3 domain containing 1 (SORBS1); and C21orf63 (see EXAMPLE II below.

Likewise, the validated up-regulated genes include: Erg-2, MARCKS-like protein (MLP); SRY (sex determining region Y)-box 4 (SOX4); Fatty acid binding protein 5 (FABP5); and MAL2.

Additionally, the mRNA expression levels of the ZNF185, FLJ14084, SVIL, KIAA1210, PRIMA1 and TU3A genes in prostate cancer cell lines were restored by treatment of cells with 5-aza-2'-deoxycytidine, an inhibitor of DNA methylation, thereby implicating the transcriptional silencing of these genes by methylation in prostate cancer cells, and indicating that genomic DNA methylation is correlated with prostate tumorigenesis.

Methylation-specific PCR even further confirmed methylation of the 5'CpG islands of the ZNF185 gene in all metastatic tissues and 44% of the localized tumor tissues as well as in the

prostate cancer cell lines tested. Thus, transcriptional silencing of particular inventive markers, including ZNF185, by DNA methylation in prostate tumor tissues is correlated with prostate tumorigenesis and progression.

Various aspects of the present invention provide one or more gene markers, or panels thereof, whereby at least one of expression, and methylation analysis of one or a combination of the members of the panel enables the detection of cell proliferative disorders of the prostate with a particularly high sensitivity, specificity and/or predictive value. The inventive testing methods have particular utility for the screening of at-risk populations. The inventive methods have advantages over prior art methods, because of improved sensitivity, specificity and likely patient compliance.

The present invention provides novel methods for detecting or distinguishing between prostate cell proliferative disorders..

One embodiment the invention provides a method for detecting and/or for detecting and distinguishing between or among prostate cell proliferative disorders in a subject. Said method comprises: i) contacting genomic DNA isolated from a test sample obtained from the subject with at least one reagent, or series of reagents that distinguishes between methylated and non-methylated CpG dinucleotides within at least one target region of the genomic DNA, wherein the nucleotide sequence of said target region comprises at least one CpG dinucleotide sequence; and ii) detecting, or detecting and distinguishing between or among prostate cell proliferative disorders based on determination of the corresponding genomic methylation state.

Another embodiment the method comprises the use of one or more genes or genomic sequences selected from the group consisting of: (ZNF185), bullous pemphigoid antigen gene (BPAG1), prostate secretory protein (PSP94), supervillin (SVIL); proline rich membrane anchor 1 (PRIMA1); TU3A; FLJ14084; KIAA1210; sorbin and SH3 domain containing 1 (SORBS1), C21orf63, Erg-2, MARCKS-like protein (MLP); SRY (sex determining region Y)-box 4 (SOX4); Fatty acid binding protein 5 (FABP5); and MAL2.as markers for the differentiation, detection and distinguishing of prostate cell proliferative disorders and cancer.

Said use of the gene may be enabled by means of any analysis of the expression of the gene, by means of mRNA expression analysis or protein expression analysis. However, in the most preferred embodiment of the invention, the detection, differentiation and distinguishing of colorectal cell proliferative disorders is enabled by means of analysis of the *methylation status* of one or more genes or genomic sequences selected from the group consisting of: (ZNF185), bullous pemphigoid antigen gene (BPAG1), prostate secretory protein (PSP94), supervillin (SVIL); proline rich membrane anchor 1 (PRIMA1); TU3A; FLJ14084; KIAA1210; sorbin and SH3 domain containing 1

(SORBS1), C21orf63, Erg-2, MARCKS-like protein (MLP); SRY (sex determining region Y)-box 4 (SOX4); Fatty acid binding protein 5 (FABP5); and MAL2 (and their regulatory and promoter elements) as markers for the differentiation, detection and distinguishing of prostate cell proliferative disorders and cancer.

The present invention provides a method for ascertaining genetic and/or epigenetic parameters of genomic DNA. The method has utility for the improved diagnosis, treatment and monitoring of prostate cell proliferative disorders, more specifically by enabling the improved identification of and differentiation between subclasses of said disorder or stages of prostate tumors.

Preferably, the source of the test sample is selected from the group consisting of cells or cell lines, histological slides, biopsies, paraffin-embedded tissue, bodily fluids, ejaculate, stool, urine, blood, and combinations thereof.

Specifically, the present invention provides a method for detecting prostate cell proliferative disorders, comprising: obtaining a biological sample comprising genomic nucleic acid(s); contacting the nucleic acid(s), or a fragment thereof, with one reagent or a plurality of reagents sufficient for distinguishing between methylated and non methylated CpG dinucleotide sequences within a target sequence of the subject nucleic acid, wherein the target sequence comprises, or hybridizes under stringent conditions to, a sequence comprising at least 16 contiguous nucleotides of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, said contiguous nucleotides comprising at least one CpG dinucleotide sequence; and determining, based at least in part on said distinguishing, the methylation state of at least one target CpG dinucleotide sequence, or an average, or a value reflecting an average methylation state of a plurality of target CpG dinucleotide sequences. Preferably, distinguishing between methylated and non methylated CpG dinucleotide sequences within the target sequence comprises methylation state-dependent conversion or non-conversion of at least one such CpG dinucleotide sequence to the corresponding converted or non-converted dinucleotide sequence.

Additional embodiments provide a method for the detection of prostate cell proliferative disorders, comprising: obtaining a biological sample having subject genomic DNA; extracting the genomic DNA; treating the genomic DNA, or a fragment thereof, with one or more reagents to convert 5-position unmethylated cytosine bases to uracil or to another base that is detectably dissimilar to cytosine in terms of hybridization properties; contacting the treated genomic DNA, or the treated fragment thereof, with an amplification enzyme and at least two primers comprising, in each case a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group

consisting of the bisulfite converted sequences corresponding to SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, wherein the treated DNA or the fragment thereof is either amplified to produce an amplificate, or is not amplified; and determining, based on a presence or absence of, or on a property of said amplificate, the methylation state of at least one CpG dinucleotide sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, or an average, or a value reflecting an average methylation state of a plurality of CpG dinucleotide sequences thereof. Preferably, at least one such hybridizing nucleic acid molecule or peptide nucleic acid molecule is bound to a solid phase.

Further embodiments provide a method for the analysis of prostate cell proliferative disorders, comprising: obtaining a biological sample having subject genomic DNA; extracting the genomic DNA; contacting the genomic DNA, or a fragment thereof, comprising one or more sequences selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, or a sequence that hybridizes under stringent conditions thereto, with one or more methylation-sensitive restriction enzymes, wherein the genomic DNA is either digested thereby to produce digestion fragments, or is not digested thereby; and determining, based on a presence or absence of, or on property of at least one such fragment, the methylation state of at least one CpG dinucleotide sequence of one or more sequences selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, or an average, or a value reflecting an average methylation state of a plurality of CpG dinucleotide sequences thereof. Preferably, the digested or undigested genomic DNA is amplified prior to said determining.

Additional embodiments provide novel genomic and chemically modified nucleic acid sequences, as well as oligonucleotides and/or PNA-oligomers for analysis of cytosine methylation patterns within sequences from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows expression of 50 significantly regulated genes in 36 prostate tissue samples (the text of Figure 1 is reproduced in TABLE 4). Cluster diagram depicting genes that distinguish metastatic (Met; n=5) from confined tumors with Gleason score 9 lymph node positive (9P; n=6) or negative (9N; n=6) and Gleason score 6 lymph node positive (6P; n=6) or negative (6N; n=5) prostate cancer and adjacent benign tissues (ABT; n=8) (n represents the number of tissues). Each row represents a gene and each column a tissue sample. Red and green represent up regulation and down regulation, respectively, relative to the median of the reference pool. Gray represents

technically inadequate or missing date, and black represents equal expression relative to the reference samples. Color saturation is proportional to the magnitude of the difference from the mean. Each gene is labeled by its gene name. Mean and standard deviation (S.D.) of the fold change in the expression levels of genes compared to ABT is shown.

Figure 2a shows forward primer (FP), reverse primer (RP) and probes used for Taqman real-time PCR.

Figure 2b shows expression levels of genes ZNF185, PSP94, BPAG1 and Erg-2 as validated by Taqman real-time PCR in 36 samples (28 cancer and 8 benign) used for analysis and an additional 8 samples (4 cancer and 4 benign). Values are expressed as the copy number of the gene relative to GAPDH levels. Metastatic tissues (Met υ) n=5, Gleason score 9, lymph node positive (9P ■) n=7 or negative (9N □) n=8 and Gleason score 6, lymph node positive (6P λ) n=6 or negative tissues (6N ○) n=6 and adjacent benign tissues (ABT σ) n=12 were used. (n represents the number of tissues). Mean ± standard deviation (S.D.) of relative expression levels of each group is shown on the left.

Figure 3a shows expression of ZNF185 levels in prostate cancer cells treated with 6 μM 5-Aza-CdR for 6 days. Four separate experiments are represented, and the error bars denote the standard deviation. The symbol "*" Indicates statistical significance over the untreated cells (p<0.05%).

Figure 3b shows the PCR primers (forward primer [FP], reverse primer [RP]), used for MSP of prostate tissues. The symbol "W" represents unmodified or wild type primers, "M," methylated-specific primers, and "U," unmethylated-specific primers. Sequence difference between modified primers and unmodified DNA are in boldface type and differences between methylated/modified and unmethylated/modified are underlined.

Figure 3c shows MSP analysis of ZNF185 DNA in prostate tissue samples and cell lines, with and without 5-Aza-CdR treatment. The amplified products were directly loaded onto DNA 500 lab chip and analyzed on Agilent 2100 Bioanalyzer. Molecular size marker is shown at left. All DNA samples were bisulfite-treated except those designated untreated. The experiments were repeated twice and the representative band of the PCR product in lanes U, M and W indicates the presence of unmethylated, methylated and wild type ZNF185 DNA, respectively.

Figure 3d shows a summary of the incidence of methylation of ZNF185 DNA in prostate tissues analyzed by MSP.

Figures 4-14 show, respectively, the expression levels of eleven genes (PRIMA1, TU3A, KIAA1210, FLJ14084; SVIL, SORBS1, C21orf63, MAL2, FABP5, SOX4 and MLP) as validated

by Taqman real-time PCR analysis (including the Kruskal-Wallis global test) in 40 prostate tissue samples and expressed as the relative fold increase (MAL2, FABP5, SOX4 and MLP) or decrease (PRIMA1, TU3A, KIAA1210, FLJ14084; SVIL, SORBS1 and C21orf63) in the mRNA expression over the adjacent benign tissues after normalization to the house-keeping gene GAPDH mRNA levels. Mean and standard deviations are shown on the right. This real-time PCR data validates results from the instant -based expression analysis. . A significant decrease in the expression of the PRIMA1, TU3A, KIAA1210, FLJ14084; SVIL, SORBS1 and C21orf63 genes was confirmed in metastatic *versus* organ confined and localized tumors compared to benign tissues ($p<0.0004$), and the MAL2, FABP5, SOX4 and MLP genes were confirmed to be upregulated in the expression in Gleason grade 6 and Gleason grade 9 tissues compared to the metastatic tissues.

Figures 15-19 show, respectively, for the FLJ14084, SVIL, PRIMA1, KIAA1210 and TU3A genes, enhanced expression of mRNA levels in prostate cancer cells (LAPC4, LNCaP and PC3 cell lines) treated with 6μM 5-Aza-CdR for 6 days. Four separate experiments are represented, and the error bars denote the standard deviation. The asterisk (*) indicates statistical significance over the untreated cells ($p<0.05\%$). The increase in the mRNA levels of FLJ14084, SVIL, PRIMA1, KIAA1210 and TU3A by 5-Aza-CdR indicates that the gene is silenced by methylation in prostate cancer cells.

DETAILED DESCRIPTION OF THE INVENTION

Genes expression was profiled in benign and untreated human prostate cancer tissues using oligonucleotide s. Six hundred seventy-four (674) genes with distinct (*i.e.*, differential expression relative to benign tissue) expression patterns in metastatic and confined tumors (Gleason score 6 and 9, lymph node invasive and non-invasive) were identified. Validation of expression profiles of seventeen (17) genes by quantitative PCR revealed a strong *inverse* correlation in the expression with progression of prostate cancer for: zinc finger protein (ZNF185), bullous pemphigoid antigen gene (BPAG1), prostate secretory protein (PSP94) (see EXAMPLE I below); and for supervillin (SVIL); proline rich membrane anchor 1 (PRIMA1); TU3A; FLJ14084; KIAA1210; sorbin and SH3 domain containing 1 (SORBS1); and C21orf63 (see EXAMPLE II below.

Likewise, the validated up-regulated genes include: Erg-2, MARCKS-like protein (MLP); SRY (sex determining region Y)-box 4 (SOX4); Fatty acid binding protein 5 (FABP5); and MAL2.

Additionally, the mRNA expression levels of the ZNF185, FLJ14084, SVIL, KIAA1210, PRIMA1 and TU3A genes in prostate cancer cell lines were restored by treatment of cells with 5-aza-2'-deoxycytidine, an inhibitor of DNA methylation, thereby implicating the transcriptional

silencing of these genes by methylation in prostate cancer cells, and indicating that genomic DNA methylation is correlated with prostate tumorigenesis.

Methylation-specific PCR even further confirmed methylation of the 5'CpG islands of the ZNF185 gene in all metastatic tissues and 44% of the localized tumor tissues as well as in the prostate cancer cell lines tested. Thus, transcriptional silencing of particular inventive markers, including ZNF185, by DNA methylation in prostate tumor tissues is correlated with prostate tumorigenesis and progression.

DEFINITIONS:

"ZNF185" (SEQ ID NOS:1 and 2) refers to the zinc finger protein 185 nucleic acid sequence (NM_007150; Y09538) and protein, and additionally includes functional variants (including conservative amino acid sequence variants as described herein), fragments, muteins, derivatives and fusion proteins thereof;

"PSP94" (SEQ ID NOS:29 and 30) refers to Prostate secretory protein 94 PSP94 nucleic acid (NM_002443; Homo sapiens microseminoprotein, beta- (MSMB), transcript variant PSP94) and protein, and additionally includes functional variants (including conservative amino acid sequence variants as described herein), fragments, muteins, derivatives and fusion proteins thereof;

"BPAG1" (SEQ ID NO:31) refers to Bullous pemphigoid antigen 1 nucleic acid (HUMBPAG1A; M69225; Human bullous pemphigoid antigen (BPAG1)) and protein, and additionally includes functional variants (including conservative amino acid sequence variants as described herein), fragments, muteins, derivatives and fusion proteins thereof;

"Erg-2" (SEQ ID NOS: 51 and 52) refers to Homo sapiens v-ets erythroblastosis virus E26 oncogene like (avian) (ERG), transcript variant 2 nucleic acid (NM_004449) and protein, and additionally includes functional variants (including conservative amino acid sequence variants as described herein), fragments, muteins, derivatives and fusion proteins thereof;

"SVIL" (SEQ ID NOS:35 and 36) refers to supervillin (SVIL) nucleic acid (AF051851.1; Homo sapiens supervillin) and protein, and additionally includes functional variants (including conservative amino acid sequence variants as described herein), fragments, muteins, derivatives and fusion proteins thereof;

"PRIMA1" (SEQ ID NO:37) refers to proline rich membrane anchor 1 (PRIMA1) nucleic acid (AI823645) and protein, and additionally includes functional variants (including conservative amino acid sequence variants as described herein), fragments, muteins, derivatives and fusion proteins thereof;

"TU3A" (SEQ ID NOS:40 and 41) refers to Homo sapiens nucleic acid (mRNA; cDNA DKFZp564N0582, from clone DKFZp564N0582) (AL050264) and protein, and additionally includes functional variants (including conservative amino acid sequence variants as described herein), fragments, muteins, derivatives and fusion proteins thereof;

"FLJ14084" (SEQ ID NOS:38 and 39) refers to FLJ14084 nucleic acid (NM_021637) and protein, and additionally includes functional variants (including conservative amino acid sequence variants as described herein), fragments, muteins, derivatives and fusion proteins thereof;

"KIAA1210" (SEQ ID NO:42) refers to the EST corresponding to AI610999;

"SORBS1" (SEQ ID NOS:32 and 33) refers to sorbin and SH3 domain containing 1 (SORBS1) nucleic acid (NM_015385; Homo sapiens sorbin and SH3 domain containing 1 (SORBS1)) and protein, and additionally includes functional variants (including conservative amino acid sequence variants as described herein), fragments, muteins, derivatives and fusion proteins thereof;

"C21orf63" (SEQ ID NO:34)refers to the EST C21ORF63; AI744591;

"MLP" (SEQ ID NOS:45 and 46) refers to Homo sapiens macrophage myristoylated alanine-rich C kinase substrate(MACMARCKS); MARCKS-like protein (MLP) nucleic acid (NM_023009.1) and protein, and additionally includes functional variants (including conservative amino acid sequence variants as described herein), fragments, muteins, derivatives and fusion proteins thereof;

"SOX4" (SEQ ID NOS:43 and 44) refers to Homo sapiens SRY (sex determining region Y)-box 4 (SOX4) nucleic acid (NM_003107) and protein, and additionally includes functional variants (including conservative amino acid sequence variants as described herein), fragments, muteins, derivatives and fusion proteins thereof;

"FABP5" (SEQ ID NOS:47 and 48) refers to Homo sapiens fatty acid binding protein 5 (FABP5) (psoriasis-associated) nucleic acid (NM_001444.1) and protein, and additionally includes functional variants (including conservative amino acid sequence variants as described herein), fragments, muteins, derivatives and fusion proteins thereof;

"MAL2" (SEQ ID NOS:49 and 50) refers to Homo sapiens mal, T-cell differentiation protein 2 (MAL2), or to Homo sapiens MAL2 proteolipid (MAL2) nucleic acid (NM_052886; AY007723) and protein, and additionally includes functional variants (including conservative amino acid sequence variants as described herein), fragments, muteins, derivatives and fusion proteins thereof;

The terms "LNCaP," "PC3" and "LAPC4" refer to the respective art-recognized human prostate cancer cell lines. Specifically, the human prostate cancer cell lines LNCaP, PC3 are from

American Type Culture Collection, Rockville, MD, USA, and LAPC4 was a gift from Dr. Charles L. Sawyers, University of California, Los Angeles, CA;

The term "Observed/Expected Ratio" ("O/E Ratio") refers to the frequency of CpG dinucleotides within a particular DNA sequence, and corresponds to the [number of CpG sites / (number of C bases × number of G bases)] × band length for each fragment;

The term "CpG island" refers to a contiguous region of genomic DNA that satisfies the criteria of (1) having a frequency of CpG dinucleotides corresponding to an "Observed/Expected Ratio" >0.6, and (2) having a "GC Content" >0.5. CpG islands are typically, but not always, between about 0.2 to about 1 kb, or to about 2kb in length;

The term "methylation state" or "methylation status" refers to the presence or absence of 5-methylcytosine ("5-mCyt") at one or a plurality of CpG dinucleotides within a DNA sequence. Methylation states at one or more particular palindromic CpG methylation sites (each having two CpG CpG dinucleotide sequences) within a DNA sequence include "unmethylated," "fully-methylated" and "hemi-methylated";

The term "hemi-methylation" or "hemimethylation" refers to the methylation state of a palindromic CpG methylation site, where only a single cytosine in one of the two CpG dinucleotide sequences of the palindromic CpG methylation site is methylated (*e.g.*, 5'-CC^{M}GG-3' (top strand): 3'-GGCC-5' (bottom strand));

The term "hypermethylation" refers to the average methylation state corresponding to an *increased* presence of 5-mCyt at one or a plurality of CpG dinucleotides within a DNA sequence of a test DNA sample, relative to the amount of 5-mCyt found at corresponding CpG dinucleotides within a normal control DNA sample;

The term "hypomethylation" refers to the average methylation state corresponding to a *decreased* presence of 5-mCyt at one or a plurality of CpG dinucleotides within a DNA sequence of a test DNA sample, relative to the amount of 5-mCyt found at corresponding CpG dinucleotides within a normal control DNA sample;

The term "" refers broadly to both "DNA s," and 'DNA chip(s),' as recognized in the art, encompasses all art-recognized solid supports, and encompasses all methods for affixing nucleic acid molecules thereto or synthesis of nucleic acids thereon;

"Genetic parameters" are mutations and polymorphisms of genes and sequences further required for their regulation. To be designated as mutations are, in particular, insertions, deletions, point mutations, inversions and polymorphisms and, particularly preferred, SNPs (single nucleotide polymorphisms);

"Epigenetic parameters" are, in particular, cytosine methylations. Further epigenetic parameters include, for example, the acetylation of histones which, however, cannot be directly analyzed using the described method but which, in turn, correlate with the DNA methylation;

The term "bisulfite reagent" refers to a reagent comprising bisulfite, disulfite, hydrogen sulfite or combinations thereof, useful as disclosed herein to distinguish between methylated and unmethylated CpG dinucleotide sequences;

The term "Methylation assay" refers to any assay for determining the methylation state of one or more CpG dinucleotide sequences within a sequence of DNA;

The term "MS.AP-PCR" (Methylation-Sensitive Arbitrarily-Primed Polymerase Chain Reaction) refers to the art-recognized technology that allows for a global scan of the genome using CG-rich primers to focus on the regions most likely to contain CpG dinucleotides, and described by Gonzalgo et al., *Cancer Research* 57:594-599, 1997;

The term "MethyLight™" refers to the art-recognized fluorescence-based real-time PCR technique described by Eads et al., *Cancer Res.* 59:2302-2306, 1999;

The term "HeavyMethyl™" assay, in the embodiment thereof implemented herein, refers to an assay, wherein methylation specific *blocking* probes (also referred to herein as *blockers*) covering CpG positions between, or covered by the amplification primers enable methylation-specific selective amplification of a nucleic acid sample;

The term "Ms-SNuPE" (Methylation-sensitive Single Nucleotide Primer Extension) refers to the art-recognized assay described by Gonzalgo & Jones, *Nucleic Acids Res.* 25:2529-2531, 1997;

The term "MSP" (Methylation-specific PCR) refers to the art-recognized methylation assay described by Herman et al. *Proc. Natl. Acad. Sci. USA* 93:9821-9826, 1996, and by US Patent No. 5,786,146;

The term "COBRA" (Combined Bisulfite Restriction Analysis) refers to the art-recognized methylation assay described by Xiong & Laird, *Nucleic Acids Res.* 25:2532-2534, 1997;

The term "MCA" (Methylated CpG Island Amplification) refers to the methylation assay described by Toyota et al., *Cancer Res.* 59:2307-12, 1999, and in WO 00/26401A1;

The term "hybridization" is to be understood as a bond of an oligonucleotide to a complementary sequence along the lines of the Watson-Crick base pairings in the sample DNA, forming a duplex structure; and

"Stringent hybridization conditions," as defined herein, involve hybridizing at 68°C in 5x SSC/5x Denhardt's solution/1.0% SDS, and washing in 0.2x SSC/0.1% SDS at room temperature, or involve the art-recognized equivalent thereof (e.g., conditions in which a hybridization is carried

out at 60°C in 2.5 x SSC buffer, followed by several washing steps at 37°C in a low buffer concentration, and remains stable). Moderately stringent conditions, as defined herein, involve including washing in 3x SSC at 42°C, or the art-recognized equivalent thereof. The parameters of salt concentration and temperature can be varied to achieve the optimal level of identity between the probe and the target nucleic acid. Guidance regarding such conditions is available in the art, for example, by Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, N.Y.; and Ausubel et al. (eds.), 1995, Current Protocols in Molecular Biology, (John Wiley & Sons, N.Y.) at Unit 2.10.

A conservative amino acid change, as is known in the relevant art, refers to a substitution of one of a family of amino acids which are related in their side chains. Naturally occurring amino acids are generally divided into four families: acidic (aspartate, glutamate), basic (lysine, arginine, histidine), non-polar (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), and uncharged polar (glycine, asparagine, glutamine, cystine, serine, threonine, tyrosine) amino acids. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. It is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have an effect on the biological properties of the resulting protein or polypeptide variant.

All references cited herein are thereby incorporated herein in their entirety.

OVERVIEW

According to EXAMPLE I below, the present invention provides, *inter alia*, biologically and clinical relevant clusters of genes characteristic of prostate cancer versus benign tissues and confined versus metastatic prostate cancer using oligonucleotide s. In EXAMPLE I, expression profiles were generated from 5 metastatic prostate tissues, and 23 confined tumors including 12 Gleason score 9 (high grade), and 11 Gleason score 6 (intermediate grade) tumors. In addition, 8 adjacent benign prostatic tissues were also studied. In EXAMPLE I, fifty (50) genes have been identified herein with distinct expression patterns in prostate cancer compared with benign prostatic tissues. Expression levels of prostate secretory protein (PSP94), zinc finger protein (ZNF185), bullous pemphigoid antigen gene (BPAG1), prostate specific transglutaminase gene (TGM4), Erg isoform 2 (Erg-2) and Rho GDP dissociation inhibitor (RhoGD-β) were validated by Taqman quantitative real-time PCR. Furthermore, analysis of the expression of ZNF185 in prostate cancer cell lines revealed

an increase in the expression by treatment with an inhibitor of DNA methylation, 5-aza-2'-deoxycytidine. Methylation specific PCR (MSP) indicated ZNF185 inactivation by CpG dinucleotide methylations in prostate cancer cell lines and cancer tissues. Our studies show that down-regulation of ZNF185, PSP94 and BPAG1 with epigenetic alteration of ZNF185 is highly associated with prostate cancer progression and serve as useful biomarkers for predicting progression of the cancer.

Likewise, according to EXAMPLE II below, the present invention provides, *inter alia*, biologically and clinical relevant clusters of genes characteristic of prostate cancer versus benign tissues and confined versus metastatic prostate cancer using oligonucleotide s. In EXAMPLE II, six hundred-twenty four (624) genes were shown by the analysis to have distinct expression patterns in metastatic and confined tumors (Gleason score 6 and 9, relative to benign tissues. A total of eleven (11) of these differentially expressed genes were selected and further validation by Taqman quantitative real time PCR to confirm the differential expression of genes according to the data.

The validated genes include seven (7) down-regulated genes, and four (4) up-regulated genes. Specifically, the validated down-regulated genes include: Supervillin (SVIL); Proline rich membrane anchor 1 (PRIMA1); TU3A; FLJ14084; KIAA1210; Sorbin and SH3 domain containing 1 (SORBS1); and C21orf63. The validated up-regulated genes include: MARCKS-like protein (MLP); SRY (sex determining region Y)-box 4 (SOX4); Fatty acid binding protein 5 (FABP5); and MAL2.

Validation confirmed the -based strong inverse correlation in the expression of all seven down-regulated genes (SVIL, PRIMA1, TU3A, FLJ14084; KIAA1210, SORBS1 and C21orf63) with progression of prostate cancer.

Likewise, validation confirmed the microarray-based correlation of increased expression, in Gleason grade 6 and Gleason grade 9 tissues, for all four upregulated genes (MLP, SOX4, FABP5 and MAL2).

Furthermore, the mRNA expression levels of the FLJ14084, SVIL, KIAA1210, PRIMA1 and TU3A genes in prostate cancer cell lines were restored by treatment of cells with 5-aza-2'-deoxycytidine, an inhibitor of DNA methylation, thereby implicating the transcriptional silencing of these genes by methylation in prostate cancer cells, and indicating that genomic DNA methylation is correlated with prostate tumorigenesis.

According to aspects of the present invention, the altered methylation and/or expression of these genes provide for novel diagnostic and/or prognostic assays for detection of precancerous and cancerous lesions of the prostate. The inventive compositions and methods have great utility as

independent and/or supplementary approaches to standard histopathological work-up of precancerous and cancerous lesions of the prostate.

Oligonucleotides. The present invention provides novel uses for genomic sequences selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, to the complements thereof, to the bisulfite-converted sequences thereof (see below), and to the complements of the bisulfite-converted sequences thereof. Additional embodiments provide modified variants of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, to the complements thereof, to the bisulfite-converted sequences thereof (see below), and to the complements of the bisulfite-converted sequences thereof, as well as oligonucleotides and/or PNA-oligomers for analysis of cytosine methylation patterns within SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, to the complements thereof, to the bisulfite-converted sequences thereof (see below), and to the complements of the bisulfite-converted sequences thereof.

An objective of the invention comprises analysis of the methylation state of one or more CpG dinucleotides within at least one of the genomic sequences selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, to the complements thereof, to the bisulfite-converted sequences thereof (see below), and to the complements of the bisulfite-converted sequences thereof.

The disclosed invention provides treated nucleic acids, derived from genomic SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, and from the complements thereof, wherein the treatment is suitable to convert at least one unmethylated cytosine base of the genomic DNA sequence to uracil or another base that is detectably dissimilar to cytosine in terms of hybridization. The genomic sequences in question may comprise one, or more, consecutive or random methylated CpG positions. Said treatment preferably comprises use of a reagent selected from the group consisting of bisulfite, hydrogen sulfite, disulfite, and combinations thereof. In a preferred embodiment of the invention, the objective comprises analysis of a modified nucleic acid comprising a sequence of at least 16, at least 18, at least 20, at least 25, or at least 30 contiguous nucleotide bases in length of a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, the complements thereof, the bisulfite-converted sequences thereof (see below), and the complements of the bisulfite-converted sequences thereof, wherein said sequence comprises at least one CpG, TpA or CpA dinucleotide and sequences complementary thereto. The sequences of the modified versions of the nucleic acid according to SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, the complements thereof,

are encompassed, wherein the modification of each genomic sequence results in the synthesis of a nucleic acid having a sequence that is unique and distinct from said genomic sequence as follows. For each sense strand genomic DNA, *e.g.*, SEQ ID NO:1, four converted versions are disclosed. A first version wherein "C" →"T," but "CpG" remains "CpG" (*i.e.*, corresponds to case where, for the genomic sequence, all "C" residues of CpG dinucleotide sequences are methylated and are thus not converted); a second version discloses the complement of the disclosed genomic DNA sequence (i.e. *anti*sense strand), wherein "C" →"T," but "CpG" remains "CpG" (*i.e.*, corresponds to case where, for all "C" residues of CpG dinucleotide sequences are methylated and are thus not converted). The 'upmethylated' converted sequences of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, and the complements thereof are encompassed herein. A third chemically converted version of each genomic sequences is provided, wherein "C" →"T" for all "C" residues, including those of "CpG" dinucleotide sequences (*i.e.*, corresponds to case where, for the genomic sequences, all "C" residues of CpG dinucleotide sequences are *un*methylated); a final chemically converted version of each sequence, discloses the complement of the disclosed genomic DNA sequence (i.e. *anti*sense strand), wherein "C" →"T" for all "C" residues, including those of "CpG" dinucleotide sequences (*i.e.*, corresponds to case where, for the complement (*anti*sense strand) of each genomic sequence, all "C" residues of CpG dinucleotide sequences are *un*methylated). The 'downmethylated' converted sequences of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, and of the complements thereof are additionally encompassed herein.

In an alternative preferred embodiment, such analysis comprises the use of an oligonucleotide or oligomer for detecting the cytosine methylation state within genomic or pretreated (chemically modified) DNA, corresponding to SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, and to the complements thereof. Said oligonucleotide or oligomer comprising a nucleic acid sequence having a length of at least 9, at least 15, at least 18, at least 20, at least 25, or at least 30 nucleotides which hybridizes, under moderately stringent or stringent conditions (as defined herein above), to a pretreated nucleic acid sequence, or to a genomic sequence according to SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, or to the complements thereof.

The present invention includes nucleic acid molecules (*e.g.*, oligonucleotides and peptide nucleic acid (PNA) molecules (PNA-oligomers)) that hybridize under moderately stringent and/or stringent hybridization conditions to all or a portion of the sequences SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, to the complements thereof, to the bisulfite-converted sequences thereof (see below), and to the complements of the bisulfite-converted sequences thereof.

The hybridizing portion of the hybridizing nucleic acids is typically at least 9, 15, 20, 25, 30 or 35 nucleotides in length. However, longer molecules have inventive utility, and are thus within the scope of the present invention.

Preferably, the hybridizing portion of the inventive hybridizing nucleic acids is at least 95%, or at least 98%, or 100% identical to the sequence, or to a portion thereof of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, to the complements thereof, to the bisulfite-converted sequences thereof (see below), and to the complements of the bisulfite-converted sequences thereof.

Hybridizing nucleic acids of the type described herein can be used, for example, as a primer (*e.g.*, a PCR primer), or a diagnostic and/or prognostic probe or primer. Preferably, hybridization of the oligonucleotide probe to a nucleic acid sample is performed under stringent conditions and the probe is 100% identical to the target sequence. Nucleic acid duplex or hybrid stability is expressed as the melting temperature or Tm, which is the temperature at which a probe dissociates from a target DNA. This melting temperature is used to define the required stringency conditions.

For target sequences that are related and substantially identical to the corresponding sequence of SEQ ID NO:1 (and the other SEQ ID NOS recited above) (such as allelic variants and SNPs), rather than identical, it is useful to first establish the lowest temperature at which only homologous hybridization occurs with a particular concentration of salt (*e.g.*, SSC or SSPE). Then, assuming that 1% mismatching results in a 1°C decrease in the Tm, the temperature of the final wash in the hybridization reaction is reduced accordingly (for example, if sequences having > 95% identity with the probe are sought, the final wash temperature is decreased by 5°C). In practice, the change in Tm can be between 0.5°C and 1.5°C per 1% mismatch.

Examples of inventive oligonucleotides of length X (in nucleotides), as indicated by polynucleotide positions with reference to SEQ ID NO:1, include those corresponding to sets (sense and antisense sets) of consecutively overlapping oligonucleotides of length X, where the oligonucleotides within each consecutively overlapping set (corresponding to a given X value) are defined as the finite set of Z oligonucleotides from nucleotide positions:

n to (n + (X-1));

where n=1, 2, 3,...(Y-(X-1));

where Y equals the length (nucleotides or base pairs) of SEQ ID NO:1 (3,614);

where X equals the common length (in nucleotides) of each oligonucleotide in the set (*e.g.*, X=20 for a set of consecutively overlapping 20-mers); and

where the number (Z) of consecutively overlapping oligomers of length X for a given SEQ ID NO of length Y is equal to Y-(X-1). For example Z= 3,614-19= 3,595 for either sense or antisense sets of SEQ ID NO:1, where X=20.

Preferably, the set is limited to those oligomers that comprise at least one CpG, TpG or CpA dinucleotide.

Examples of inventive 20-mer oligonucleotides include the following set of 3,595 oligomers (and the antisense set complementary thereto), indicated by polynucleotide positions with reference to SEQ ID NO:1:

1-20, 2-21, 3-22, 4-23, 5-24, ...3593-3612, 3594-3613 and 3595-3614.

Preferably, the set is limited to those oligomers that comprise at least one CpG, TpG or CpA dinucleotide.

The present invention encompasses, for SEQ ID NO:1 (sense and antisense), multiple consecutively overlapping sets of oligonucleotides or modified oligonucleotides of length X, where, *e.g.*, X= 9, 10, 17, 20, 22, 23, 25, 27, 30 or 35 nucleotides. Likewise, the invention encompasses analogous sets of oligos corresponding to SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, to the complements thereof, to the bisulfite-converted sequences thereof (see below), and to the complements of the bisulfite-converted sequences thereof.

The oligonucleotides or oligomers according to the present invention constitute effective tools useful to ascertain genetic and epigenetic parameters of the genomic sequence corresponding to SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, to the complements thereof, to the bisulfite-converted sequences thereof (see below), and to the complements of the bisulfite-converted sequences thereof. Preferred sets of such oligonucleotides or modified oligonucleotides of length X are those consecutively overlapping sets of oligomers corresponding to at least one of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, to the complements thereof, to the bisulfite-converted sequences thereof (see below), and to the complements of the bisulfite-converted sequences thereof. Preferably, said oligomers comprise at least one CpG, TpG or CpA dinucleotide.

Oligonucleotides and PNA-oligomers capable of hybridizing, as described herein above, to the various bisulfite-converted sequences of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, and to the complements of the bisulfite-converted sequences thereof are also within the scope of the present invention.

The oligonucleotides of the invention can also be modified by chemically linking the oligonucleotide to one or more moieties or conjugates to enhance the activity, stability or detection

of the oligonucleotide. Such moieties or conjugates include chromophores, fluorophors, lipids such as cholesterol, cholic acid, thioether, aliphatic chains, phospholipids, polyamines, polyethylene glycol (PEG), palmityl moieties, and others as disclosed in, for example, United States Patent Numbers 5,514,758, 5,565,552, 5,567,810, 5,574,142, 5,585,481, 5,587,371, 5,597,696 and 5,958,773. The probes may also exist in the form of a PNA (peptide nucleic acid) which has particularly preferred pairing properties. Thus, the oligonucleotide may include other appended groups such as peptides, and may include hybridization-triggered cleavage agents (Krol et al., *BioTechniques* 6:958-976, 1988) or intercalating agents (Zon, *Pharm. Res.* 5:539-549, 1988). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a chromophore, fluorophor, peptide, hybridization-triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The oligonucleotide may also comprise at least one art-recognized modified sugar and/or base moiety, or may comprise a modified backbone or non-natural internucleoside linkage.

The oligonucleotides or oligomers according to particular embodiments of the present invention are typically used in 'sets,' which contain at least one oligomer for analysis of each of the CpG dinucleotides of genomic sequences SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, to the complements thereof, or to the corresponding CpG, TpG or CpA dinucleotide within a sequence of the corresponding pretreated nucleic acids, and sequences complementary thereto. However, it is anticipated that for economic or other factors it may be preferable to analyze a limited selection of the CpG dinucleotides within said sequences, and the content of the set of oligonucleotides is altered accordingly.

Therefore, in particular embodiments, the present invention provides a set of at least two (2) (oligonucleotides and/or PNA-oligomers) useful for detecting the cytosine methylation state in pretreated genomic DNA corresponding to SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, to the complements thereof. These probes enable diagnosis, classification and/or therapy of genetic and epigenetic parameters of prostate cell proliferative disorders and tumors. The set of oligomers may also be used for detecting single nucleotide polymorphisms (SNPs) in the above-described pretreated genomic DNA, and sequences complementary thereto.

In preferred embodiments, at least one, and more preferably all members of a set of oligonucleotides is bound to a solid phase.

In further embodiments, the present invention provides a set of at least two (2) oligonucleotides that are used as 'primer' oligonucleotides for amplifying DNA sequences of one of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49 and 51, the complements thereof,

the bisulfite-converted sequences thereof (see below), or the complements of the bisulfite-converted sequences thereof.

It is anticipated that the oligonucleotides may constitute all or part of an "array" or "DNA chip" (*i.e.*, an arrangement of different oligonucleotides and/or PNA-oligomers bound to a solid phase). Such an array of different oligonucleotide- and/or PNA-oligomer sequences can be characterized, for example, in that it is arranged on the solid phase in the form of a rectangular or hexagonal lattice. The solid-phase surface may be composed of silicon, glass, polystyrene, aluminum, steel, iron, copper, nickel, silver, or gold. Nitrocellulose as well as plastics such as nylon, which can exist in the form of pellets or also as resin matrices, may also be used. An overview of the Prior Art in oligomer array manufacturing can be gathered from a special edition of Nature Genetics (*Nature Genetics Supplement*, Volume 21, January 1999, and from the literature cited therein). Fluorescently labeled probes are often used for the scanning of immobilized DNA arrays. The simple attachment of Cy3 and Cy5 dyes to the 5'-OH of the specific probe are particularly suitable for fluorescence labels. The detection of the fluorescence of the hybridized probes may be carried out, for example, via a confocal microscope. Cy3 and Cy5 dyes, besides many others, are commercially available.

It is also anticipated that the oligonucleotides, or particular sequences thereof, may constitute all or part of an "virtual array" wherein the oligonucleotides, or particular sequences thereof, are used, for example, as 'specifiers' as part of, or in combination with a diverse population of unique labeled probes to analyze a complex mixture of analytes. Such a method, for example is described in US 2003/0013091 (United States serial number 09/898,743, published 16 January 2003). In such methods, enough labels are generated so that each nucleic acid in the complex mixture (*i.e.*, each analyte) can be uniquely bound by a unique label and thus detected (each label is directly counted, resulting in a digital read-out of each molecular species in the mixture).

It is particularly preferred that the oligomers according to the invention are utilised for at least one of: detection of; detection and differentiation between or among subclasses of; diagnosis of; prognosis of; treatment of; monitoring of; and treatment and monitoring of prostate cell proliferative disorders and cancer. This is enabled by use of said sets for the detection or detection and differentiation of one or more prostate tissues as described herein.

In preferred embodiments, expression or genomic methylation state is determined by one or more methods comprising amplification of 'treated' (e.g., bisulfite-treated) DNA. The fragments obtained by means of the amplification can carry a directly or indirectly detectable label. Preferred are labels in the form of fluorescence labels, radionuclides, or detachable molecule fragments having

a typical mass which can be detected in a mass spectrometer. Where said labels are mass labels, it is preferred that the labeled amplificates have a single positive or negative net charge, allowing for better detectability in the mass spectrometer. The detection may be carried out and visualized by means of, *e.g.*, matrix assisted laser desorption/ionization mass spectrometry (MALDI) or using electron spray mass spectrometry (ESI).

Matrix Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-TOF) is a very efficient development for the analysis of biomolecules (Karas & Hillenkamp, *Anal Chem.*, 60:2299-301, 1988). An analyte is embedded in a light-absorbing matrix. The matrix is evaporated by a short laser pulse thus transporting the analyte molecule into the vapor phase in an unfragmented manner. The analyte is ionized by collisions with matrix molecules. An applied voltage accelerates the ions into a field-free flight tube. Due to their different masses, the ions are accelerated at different rates. Smaller ions reach the detector sooner than bigger ones. MALDI-TOF spectrometry is well suited to the analysis of peptides and proteins. The analysis of nucleic acids is somewhat more difficult (Gut & Beck, *Current Innovations and Future Trends*, 1:147-57, 1995). The sensitivity with respect to nucleic acid analysis is approximately 100-times less than for peptides, and decreases disproportionately with increasing fragment size. Moreover, for nucleic acids having a multiply negatively charged backbone, the ionization process via the matrix is considerably less efficient. In MALDI-TOF spectrometry, the selection of the matrix plays an eminently important role. For desorption of peptides, several very efficient matrixes have been found which produce a very fine crystallisation. There are now several responsive matrixes for DNA, however, the difference in sensitivity between peptides and nucleic acids has not been reduced. This difference in sensitivity can be reduced, however, by chemically modifying the DNA in such a manner that it becomes more similar to a peptide. For example, phosphorothioate nucleic acids, in which the usual phosphates of the backbone are substituted with thiophosphates, can be converted into a charge-neutral DNA using simple alkylation chemistry (Gut & Beck, *Nucleic Acids Res.* 23: 1367-73, 1995). The coupling of a charge tag to this modified DNA results in an increase in MALDI-TOF sensitivity to the same level as that found for peptides. A further advantage of charge tagging is the increased stability of the analysis against impurities, which makes the detection of unmodified substrates considerably more difficult.

Methylation Assay Procedures. Various methylation assay procedures are known in the art, and can be used in conjunction with the present invention. These assays allow for determination of the methylation state of one or a plurality of CpG dinucleotides (*e.g.*, CpG islands) within a DNA sequence. Such assays involve, among other techniques, DNA sequencing of bisulfite-treated DNA,

PCR (for sequence-specific amplification), Southern blot analysis, and use of methylation-sensitive restriction enzymes.

For example, genomic sequencing has been simplified for analysis of DNA methylation patterns and 5-methylcytosine distribution by using bisulfite treatment (Frommer et al., *Proc. Natl. Acad. Sci. USA* 89:1827-1831, 1992). Additionally, restriction enzyme digestion of PCR products amplified from bisulfite-converted DNA is used, *e.g.*, the method described by Sadri & Hornsby (*Nucl. Acids Res.* 24:5058-5059, 1996), or COBRA (Combined Bisulfite Restriction Analysis) (Xiong & Laird, *Nucleic Acids Res.* 25:2532-2534, 1997).

COBRA. COBRA analysis is a quantitative methylation assay useful for determining DNA methylation levels at specific gene loci in small amounts of genomic DNA (Xiong & Laird, *Nucleic Acids Res.* 25:2532-2534, 1997). Briefly, restriction enzyme digestion is used to reveal methylation-dependent sequence differences in PCR products of sodium bisulfite-treated DNA. Methylation-dependent sequence differences are first introduced into the genomic DNA by standard bisulfite treatment according to the procedure described by Frommer et al. (*Proc. Natl. Acad. Sci. USA* 89:1827-1831, 1992). PCR amplification of the bisulfite converted DNA is then performed using primers specific for the interested CpG islands, followed by restriction endonuclease digestion, gel electrophoresis, and detection using specific, labeled hybridization probes. Methylation levels in the original DNA sample are represented by the relative amounts of digested and undigested PCR product in a linearly quantitative fashion across a wide spectrum of DNA methylation levels. In addition, this technique can be reliably applied to DNA obtained from microdissected paraffin-embedded tissue samples. Typical reagents (*e.g.*, as might be found in a typical COBRA-based kit) for COBRA analysis may include, but are not limited to: PCR primers for specific gene (or methylation-altered DNA sequence or CpG island); restriction enzyme and appropriate buffer; gene-hybridization oligo; control hybridization oligo; kinase labeling kit for oligo probe; and radioactive nucleotides. Additionally, bisulfite conversion reagents may include: DNA denaturation buffer; sulfonation buffer; DNA recovery reagents or kits (*e.g.*, precipitation, ultrafiltration, affinity column); desulfonation buffer; and DNA recovery components.

Preferably, assays such as "MethyLight ™" (a fluorescence-based real-time PCR technique) (Eads et al., *Cancer Res.* 59:2302-2306, 1999), Ms-SNuPE (Methylation-sensitive Single Nucleotide Primer Extension) reactions (Gonzalgo & Jones, *Nucleic Acids Res.* 25:2529-2531, 1997), methylation-specific PCR ("MSP"; Herman et al., *Proc. Natl. Acad. Sci. USA* 93:9821-9826, 1996; US Patent No. 5,786,146), and methylated CpG island amplification ("MCA"; Toyota et al., *Cancer Res.* 59:2307-12, 1999) are used alone or in combination with other of these methods.

MethyLight ™. The MethyLight ™ assay is a high-throughput quantitative methylation assay that utilizes fluorescence-based real-time PCR (TaqMan ™) technology that requires no further manipulations after the PCR step (Eads et al., *Cancer Res.* 59:2302-2306, 1999). Briefly, the MethyLight ™ process begins with a mixed sample of genomic DNA that is converted, in a sodium bisulfite reaction, to a mixed pool of methylation-dependent sequence differences according to standard procedures (the bisulfite process converts unmethylated cytosine residues to uracil). Fluorescence-based PCR is then performed either in an "unbiased" (with primers that do not overlap known CpG methylation sites) PCR reaction, or in a "biased" (with PCR primers that overlap known CpG dinucleotides) reaction. Sequence discrimination can occur either at the level of the amplification process or at the level of the fluorescence detection process, or both.

The MethyLight ™ assay may be used as a quantitative test for methylation patterns in the genomic DNA sample, wherein sequence discrimination occurs at the level of probe hybridization. In this quantitative version, the PCR reaction provides for unbiased amplification in the presence of a fluorescent probe that overlaps a particular putative methylation site. An unbiased control for the amount of input DNA is provided by a reaction in which neither the primers, nor the probe overlie any CpG dinucleotides. Alternatively, a qualitative test for genomic methylation is achieved by probing of the biased PCR pool with either control oligonucleotides that do not "cover" known methylation sites (a fluorescence-based version of the "MSP" technique), or with oligonucleotides covering potential methylation sites.

The MethyLight ™ process can by used with a "TaqMan®" probe in the amplification process. For example, double-stranded genomic DNA is treated with sodium bisulfite and subjected to one of two sets of PCR reactions using TaqMan® probes; *e.g.*, with either biased primers and TaqMan® probe, or unbiased primers and TaqMan® probe. The TaqMan® probe is dual-labeled with fluorescent "reporter" and "quencher" molecules, and is designed to be specific for a relatively high GC content region so that it melts out at about 10°C higher temperature in the PCR cycle than the forward or reverse primers. This allows the TaqMan® probe to remain fully hybridized during the PCR annealing/extension step. As the Taq polymerase enzymatically synthesizes a new strand during PCR, it will eventually reach the annealed TaqMan® probe. The Taq polymerase 5' to 3' endonuclease activity will then displace the TaqMan® probe by digesting it to release the fluorescent reporter molecule for quantitative detection of its now unquenched signal using a real-time fluorescent detection system.

Typical reagents (*e.g.*, as might be found in a typical MethyLight ™-based kit) for MethyLight ™ analysis may include, but are not limited to: PCR primers for specific gene (or

methylation-altered DNA sequence or CpG island); TaqMan® probes; optimized PCR buffers and deoxynucleotides; and Taq polymerase.

Ms-SNuPE. The Ms-SNuPE technique is a quantitative method for assessing methylation differences at specific CpG sites based on bisulfite treatment of DNA, followed by single-nucleotide primer extension (Gonzalgo & Jones, *Nucleic Acids Res.* 25:2529-2531, 1997). Briefly, genomic DNA is reacted with sodium bisulfite to convert unmethylated cytosine to uracil while leaving 5-methylcytosine unchanged. Amplification of the desired target sequence is then performed using PCR primers specific for bisulfite-converted DNA, and the resulting product is isolated and used as a template for methylation analysis at the CpG site(s) of interest. Small amounts of DNA can be analyzed (*e.g.*, microdissected pathology sections), and it avoids utilization of restriction enzymes for determining the methylation status at CpG sites.

Typical reagents (*e.g.*, as might be found in a typical Ms-SNuPE-based kit) for Ms-SNuPE analysis may include, but are not limited to: PCR primers for specific gene (or methylation-altered DNA sequence or CpG island); optimized PCR buffers and deoxynucleotides; gel extraction kit; positive control primers; Ms-SNuPE primers for specific gene; reaction buffer (for the Ms-SNuPE reaction); and radioactive nucleotides. Additionally, bisulfite conversion reagents may include: DNA denaturation buffer; sulfonation buffer; DNA recovery regents or kit (*e.g.*, precipitation, ultrafiltration, affinity column); desulfonation buffer; and DNA recovery components.

MSP. MSP (methylation-specific PCR) allows for assessing the methylation status of virtually any group of CpG sites within a CpG island, independent of the use of methylation-sensitive restriction enzymes (Herman et al. *Proc. Natl. Acad. Sci. USA* 93:9821-9826, 1996; US Patent No. 5,786,146). Briefly, DNA is modified by sodium bisulfite converting all unmethylated, but not methylated cytosines to uracil, and subsequently amplified with primers specific for methylated versus unmethylated DNA. MSP requires only small quantities of DNA, is sensitive to 0.1% methylated alleles of a given CpG island locus, and can be performed on DNA extracted from paraffin-embedded samples. Typical reagents (*e.g.*, as might be found in a typical MSP-based kit) for MSP analysis may include, but are not limited to: methylated and unmethylated PCR primers for specific gene (or methylation-altered DNA sequence or CpG island), optimized PCR buffers and deoxynucleotides, and specific probes.

MCA. The MCA technique is a method that can be used to screen for altered methylation patterns in genomic DNA, and to isolate specific sequences associated with these changes (Toyota et al., *Cancer Res.* 59:2307-12, 1999). Briefly, restriction enzymes with different sensitivities to cytosine methylation in their recognition sites are used to digest genomic DNAs from primary

tumors, cell lines, and normal tissues prior to arbitrarily primed PCR amplification. Fragments that show differential methylation are cloned and sequenced after resolving the PCR products on high-resolution polyacrylamide gels. The cloned fragments are then used as probes for Southern analysis to confirm differential methylation of these regions. Typical reagents (*e.g.*, as might be found in a typical MCA-based kit) for MCA analysis may include, but are not limited to: PCR primers for arbitrary priming Genomic DNA; PCR buffers and nucleotides, restriction enzymes and appropriate buffers; gene-hybridization oligos or probes; control hybridization oligos or probes.

Preferred Embodiments

Particular aspects of the present invention provide a method for detecting, or for detecting and distinguishing between or among prostate cell proliferative disorders or stages thereof in a subject comprising:obtaining, from the subject, a biological sample; and determining, using a suitable assay, the expression level of at least one gene or sequence selected from the group consisting of: ZNF185 (SEQ ID NOS:1 and 2); PSP94 (SEQ ID NOS:29 and 30); BPAG1 (SEQ ID NO:31); SORBS1 (SEQ ID NOS:32 and 33); C21orf63 (SEQ ID NO:34); SVIL (SEQ ID NOS:35 and 36); PRIMA1 (SEQ ID NO:37); FLJ14084 (SEQ ID NOS:38 and 39); TU3A (SEQ ID NOS:40 and 41); KIAA1210 (SEQ ID NO:42); SOX4 (SEQ ID NOS:43 and 44); MLP (SEQ ID NOS:45 and 46); FABP5 (SEQ ID NOS:47 and 48); MAL2 (SEQ ID NOS:49 and 50); Erg-2 (SEQ ID NOS: 51 and 52); and sequences that hybridize under high stringency thereto, whereby detecting and distinguishing between or among prostate cell proliferative disorders or stages thereof is, at least in part, afforded.

Preferably, the expression level is determined by detecting the presence, absence or level of mRNA transcribed from said gene or sequence. Preferably, the expression level is determined by detecting the presence, absence or level of a polypeptide encoded by said gene or sequence. Preferably, the polypeptide is detected by at least one method selected from the group consisting of immunoassay, ELISA immunoassay, radioimmunoassay, and antibody. Preferably, the expression is determined by detecting the presence or absence of CpG methylation within said gene or sequence, wherein hypermethylation indicates the presence of, or stage of the prostate cell proliferative disorder.

Preferably, detecting and distinguishing between or among prostate cell proliferative disorders or stages thereof is, at least in part, based on a *decrease* in expression of at least one gene or sequence selected from the group consisting of: ZNF185 (SEQ ID NOS:1 and 2); PSP94 (SEQ ID NOS:29 and 30); BPAG1 (SEQ ID NO:31); SORBS1 (SEQ ID NOS:32 and 33); C21orf63 (SEQ ID NO:34); SVIL (SEQ ID NOS:35 and 36); PRIMA1 (SEQ ID NO:37); FLJ14084 (SEQ ID NOS:38 and 39); TU3A (SEQ ID NOS:40 and 41); KIAA1210 (SEQ ID NO:42); and sequences that hybridize under high stringency thereto. Preferably, and alternatively, detecting and distinguishing between or among prostate cell proliferative disorders or stages thereof is, at least in part, based on a *increase* in expression of at least one gene or sequence selected from the group consisting of: SOX4 (SEQ ID NOS:43 and 44); MLP (SEQ ID NOS:45 and 46); FABP5 (SEQ ID NOS:47 and 48); MAL2 (SEQ ID NOS:49 and 50); Erg-2 (SEQ ID NOS: 51 and 52); and sequences that hybridize under high stringency thereto.

Preferably, expression is of at least one gene or sequence selected from the group consisting of: ZNF185 (SEQ ID NOS:1 and 2); SVIL (SEQ ID NOS:35 and 36); PRIMA1 (SEQ ID NO:37); FLJ14084 (SEQ ID NOS:38 and 39); TU3A (SEQ ID NOS:40 and 41); KIAA1210 (SEQ ID NO:42); and sequences that hybridize under high stringency thereto.

Additional embodiments provide a method for detecting, or for detecting and distinguishing between or among prostate cell proliferative disorders or stages thereof in a subject, comprising: obtaining, from the subject, a biological sample having genomic DNA; and contacting genomic DNA obtained from the subject with at least one reagent, or series of reagents that distinguishes between methylated and non-methylated CpG dinucleotides within at least one target region of the genomic DNA, wherein the target region comprises, or hybridizes under stringent conditions to at least 16 contiguous nucleotides of at least one sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof, wherein said contiguous nucleotides comprise at least one CpG dinucleotide sequence, and whereby detecting, or detecting and distinguishing between or among colon cell proliferative disorders or stages thereof is, at least in part, afforded.

Preferably, normal, non-prostate cell proliferative disorders, or adjacent benign tissues are distinguished from at least one condition selected from the group consisting of: intermediate, T2,

Gleason score 6 lymph node positive and negative; high grade,T3, Gleason score 9 lymph node positive and negative; prostatic adenocarcinoma; and metastatic tumors.

Preferably, adjacent benign tissue is distinguished from at least one condition selected from the group consisting of: intermediate, T2, Gleason score 6 lymph node positive and negative; high grade,T3, Gleason score 9 lymph node positive and negative; prostatic adenocarcinoma; and metastatic tumors. Preferably, adjacent benign tissue is distinguished from at least one condition selected from the group consisting of: intermediate, T2, Gleason score 6 lymph node positive and negative; high grade,T3, Gleason score 9 lymph node positive and negative; prostatic adenocarcinoma; and metastatic tumors, and the target region comprises, or hybridizes under stringent conditions to at least 16 contiguous nucleotides of a sequence selected from the group consisting of ZNF185 (SEQ ID NO:1); PSP94 (SEQ ID NO:29); BPAG1 (SEQ ID NO:31); SORBS1 (SEQ ID NO:32); C21orf63 (SEQ ID NO:34); SVIL (SEQ ID NS:35); PRIMA1 (SEQ ID NO:37); FLJ14084 (SEQ ID NO:38); TU3A (SEQ ID NO:40); KIAA1210 (SEQ ID NO:42); and sequences complementary thereto. Preferably, adjacent benign tissue is distinguished from at least one condition selected from the group consisting of: intermediate, T2, Gleason score 6 lymph node positive and negative; high grade,T3, Gleason score 9 lymph node positive and negative; prostatic adenocarcinoma; and metastatic tumors, and the target region comprises, or hybridizes under stringent conditions to at least 16 contiguous nucleotides of a sequence selected from the group consisting of ZNF185 (SEQ ID NO:1); SVIL (SEQ ID NO:35); PRIMA1 (SEQ ID NO:37); FLJ14084 (SEQ ID NO:38); TU3A (SEQ ID NO:40); KIAA1210 (SEQ ID NO:42); and sequences complementary thereto.

In alternate preferred embodiments, tissues originating from the prostate are distinguished from tissues of non-prostate origin. Preferably, prostate cell proliferative disorders are distinguished from healthy tissues, and the target region comprises, or hybridizes under stringent conditions to at least 16 contiguous nucleotides of a sequence selected from the group consisting of ZNF185 (SEQ ID NO:1); PSP94 (SEQ ID NO:29); BPAG1 (SEQ ID NO:31); SORBS1 (SEQ ID NO:32); C21orf63 (SEQ ID NO:34); SVIL (SEQ ID NO:35); PRIMA1 (SEQ ID NO:37); FLJ14084 (SEQ ID NO:38); TU3A (SEQ ID NO:40); KIAA1210 (SEQ ID NO:42); and sequences complementary thereto.

Yet further embodiments provide a method for detecting, or for detecting and distinguishing between or among prostate cell proliferative disorders or stages thereof in a subject, comprising: obtaining, from a subject, a biological sample having genomic DNA; contacting the genomic DNA, or a fragment thereof, with one reagent or a plurality of reagents that distinguishes between methylated and non methylated CpG dinucleotide sequences within at least one target sequence of the genomic DNA, or fragment thereof, wherein the target sequence comprises, or hybridizes under stringent conditions to, at least 16 contiguous nucleotides of a sequence taken from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof, said contiguous nucleotides comprising at least one CpG dinucleotide sequence; and determining, based at least in part on said distinguishing, the methylation state of at least one target CpG dinucleotide sequence, or an average, or a value reflecting an average methylation state of a plurality of target CpG dinucleotide sequences, whereby detecting, or detecting and distinguishing between or among prostate cell proliferative disorders or stages thereof is, at least in part, afforded.

Preferably, detecting, or detecting and distinguishing between or among prostate cell proliferative disorders or stages thereof comprises detecting, or detecting and distinguishing between or among one or more tissues selected from the group consisting of: adjacent benign tissues; intermediate, T2, Gleason score 6 lymph node positive or negative tissue; high grade, T3, Gleason score 9 lymph node positive or negative tissue; prostatic adenocarcinoma; and metastatic tumors.

Preferably, distinguishing between methylated and non methylated CpG dinucleotide sequences within the target sequence comprises converting unmethylated cytosine bases within the target sequence to uracil or to another base that is detectably dissimilar to cytosine in terms of hybridization properties. Preferably, distinguishing between methylated and non methylated CpG dinucleotide sequences within the target sequence(s) comprises methylation state-dependent conversion or non-conversion of at least one CpG dinucleotide sequence to the corresponding converted or non-converted dinucleotide sequence.

Preferably, the biological sample is selected from the group consisting of cell lines, histological slides, biopsies, paraffin-embedded tissue, bodily fluids, ejaculate, urine, blood, and combinations thereof.

Preferably, distinguishing between methylated and non methylated CpG dinucleotide sequences within the target sequence comprises use of at least one nucleic acid molecule or peptide nucleic acid (PNA) molecule comprising, in each case a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under stringent conditions to a bisulfite-converted sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof. Preferably, the contiguous sequence comprises at least one CpG, TpG or CpA dinucleotide sequence. Preferably, at least two such nucleic acid molecules, or peptide nucleic acid (PNA) molecules are used. Preferably, at least two such nucleic acid molecules are used as primer oligonucleotides for the amplification of a bisulfite-converted sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51; sequences that hybridize under stringent conditions therto; and complements thereof. Preferably, at least four such nucleic acid molecules, peptide nucleic acid (PNA) molecules are used.

Further embodiments provide a method for detecting, or detecting and distinguishing between or among prostate cell proliferative disorders or stages thereof in a subject, comprising: obtaining, from a subject, a biological sample having genomic DNA; extracting or otherwise isolating the genomic DNA; treating the genomic DNA, or a fragment thereof, with one or more reagents to convert cytosine bases that are unmethylated in the 5-position thereof to uracil or to another base that is detectably dissimilar to cytosine in terms of hybridization properties; contacting the treated genomic DNA, or the treated fragment thereof, with an amplification enzyme and at least two primers comprising, in each case a contiguous sequence of at least 9 nucleotides that is complementary to, or hybridizes under stringent conditions to a bisulfite-converted sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof, wherein the treated genomic DNA or the fragment thereof is either amplified to produce at least one amplificate, or is not amplified; and determining, based on a presence or absence of, or on a property of said amplificate, the methylation state of at least one CpG dinucleotide of a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof, or an average, or a value reflecting an average methylation state of a plurality of said CpG dinucleotides, whereby at least one

of detecting, and detecting and distinguishing between prostate cell proliferative disorders or stages thereof is, at least in part, afforded.

Preferably, treating the genomic DNA, or the fragment thereof comprises use of a reagent selected from the group consisting of bisulfite, hydrogen sulfite, disulfite, and combinations thereof.

Preferably, contacting or amplifying comprises use of at least one method selected from the group consisting of: use of a heat-resistant DNA polymerase as the amplification enzyme; use of a polymerase lacking 5'-3' exonuclease activity; use of a polymerase chain reaction (PCR); generation of a amplificate nucleic acid molecule carrying a detectable labels; and combinations thereof.

Preferably, the detectable amplificate label is selected from the label group consisting of: fluorescent labels; radionuclides or radiolabels; amplificate mass labels detectable in a mass spectrometer; detachable amplificate fragment mass labels detectable in a mass spectrometer; amplificate, and detachable amplificate fragment mass labels having a single-positive or single-negative net charge detectable in a mass spectrometer; and combinations thereof.

Preferably, the biological sample obtained from the subject is selected from the group consisting of cell lines, histological slides, biopsies, paraffin-embedded tissue, bodily fluids, ejaculate, urine, blood, and combinations thereof.

Preferably, detecting, or detecting and distinguishing between or among prostate cell proliferative disorders or stages thereof comprises detecting, or detecting and distinguishing between or among one or more tissues selected from the group consisting of: adjacent benign tissues; intermediate, T2, Gleason score 6 lymph node positive or negative tissue; high grade, T3, Gleason score 9 lymph node positive or negative tissue; prostatic adenocarcinoma; and metastatic tumors.

Preferably, the method further comprises, for the step of contacting the treated genomic DNA, the use of at least one nucleic acid molecule or peptide nucleic acid molecule comprising in each case a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under stringent conditions to a bisulfite-converted sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof, wherein said nucleic acid molecule or peptide nucleic acid molecule suppresses amplification of the nucleic acid to which it is hybridized.

Preferably, the nucleic acid molecule or peptide nucleic acid molecule is in each case modified at the 5'-end thereof to preclude degradation by an enzyme having 5'-3' exonuclease activity. Preferably, the nucleic acid molecule or peptide nucleic acid molecule is in each case lacking a 3' hydroxyl group. Preferably, the amplification enzyme is a polymerase lacking 5'-3' exonuclease activity.

Preferably, "determining" comprises hybridization of at least one nucleic acid molecule or peptide nucleic acid molecule in each case comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under stringent conditions to a bisulfite-converted sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof. Preferably, at least one such hybridizing nucleic acid molecule or peptide nucleic acid molecule is bound to a solid phase. Preferably, a plurality of such hybridizing nucleic acid molecules or peptide nucleic acid molecules are bound to a solid phase in the form of a nucleic acid or peptide nucleic acid array selected from the array group consisting of linear or substantially so, hexagonal or substantially so, rectangular or substantially so, and combinations thereof.

Preferably, the method further comprises extending at least one such hybridized nucleic acid molecule by at least one nucleotide base. Preferably, "determining" comprises sequencing of the amplificate. Preferably, "contacting" or amplifying comprises use of methylation-specific primers.

Preferably, for the "contacting" step, primer oligonucleotides comprising one or more CpG; TpG or CpA dinucleotidesn are used; and the method further comprises, for the determining step, the use of at least one method selected from the group consisting of: hybridizing in at least one nucleic acid molecule or peptide nucleic acid molecule comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under stringent conditions to a bisulfite-converted sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof; hybridizing at least one nucleic acid molecule that is bound to a solid phase and comprises a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under stringent conditions to a bisulfite-converted sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements

thereof; hybridizing at least one nucleic acid molecule comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under stringent conditions to a bisulfite-converted sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof, and extending at least one such hybridized nucleic acid molecule by at least one nucleotide base; and sequencing, in the determining step, of the amplificate.

Preferably, for the contacting step, uat least one nucleic acid molecule or peptide nucleic acid molecule is used, comprising in each case a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under stringent conditions to a bisulfite-converted sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof, wherein said nucleic acid molecule or peptide nucleic acid molecule suppresses amplification of the nucleic acid to which it is hybridized; and the method further comprises, in the determining step, the use of at least one method selected from the group consisting of: hybridizing in at least one nucleic acid molecule or peptide nucleic acid molecule comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under stringent conditions to a bisulfite-converted sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof; hybridizing at least one nucleic acid molecule that is bound to a solid phase and comprises a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under stringent conditions to a bisulfite-converted sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof; hybridizing at least one nucleic acid molecule comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under stringent conditions to a bisulfite-converted sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof, and extending at least one such hybridized nucleic acid molecule by at least one nucleotide base; and sequencing, in the determining step, of the amplificate.

Preferably, the method comprises, in the "contacting" step, amplification by primer oligonucleotides comprising one or more CpG; TpG or CpA dinucleotides, and further comprises, in

the "determining" step, hybridizing at least one detectably labeled nucleic acid molecule comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under stringent conditions to a bisulfite-converted sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof.

Preferably, the method comprises, in the "contacting" step, the use of at least one nucleic acid molecule or peptide nucleic acid molecule comprising in each case a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under stringent conditions to a bisulfite-converted sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof, wherein said nucleic acid molecule or peptide nucleic acid molecule suppresses amplification of the nucleic acid to which it is hybridized, and further comprises, in the "determining" step, hybridizing at least one detectably labeled nucleic acid molecule comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under stringent conditions to a bisulfite-converted sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof.

Yet additional embodiments provide a method for detecting, or for detecting and distinguishing between or among prostate cell proliferative disorders or stages thereof in a subject, comprising: obtaining, from a subject, a biological sample having genomic DNA; extracting, or otherwise isolating the genomic DNA; contacting the genomic DNA, or a fragment thereof, comprising at least 16 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, complements thereof; and sequences that hybridize under stringent conditions thereto, with one or more methylation-sensitive restriction enzymes, wherein the genomic DNA is, with respect to each cleavage recognition motif thereof, either cleaved thereby to produce cleavage fragments, or not cleaved thereby; and determining, based on a presence or absence of, or on property of at least one such cleavage fragment, the methylation state of at least one CpG dinucleotide of a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51; and complements thereof, or an average, or a value reflecting an average methylation state of a plurality

of said CpG dinucleotides, whereby at least one of detecting, or of detecting and differentiating between or among prostate cell proliferative disorders or stages thereof is, at least in part, afforded.

Preferably, the method further comprises, prior to determining, amplifying of the digested or undigested genomic DNA. Preferably, amplifying comprises use of at least one method selected from the group consisting of: use of a heat resistant DNA polymerase as an amplification enzyme; use of a polymerase lacking 5'-3' exonuclease activity; use of a polymerase chain reaction (PCR); generation of a amplificate nucleic acid carrying a detectable label; and combinations thereof.

Preferalby, the detectable amplificate label is selected from the label group consisting of: fluorescent labels; radionuclides or radiolabels; amplificate mass labels detectable in a mass spectrometer; detachable amplificate fragment mass labels detectable in a mass spectrometer; amplificate, and detachable amplificate fragment mass labels having a single-positive or single-negative net charge detectable in a mass spectrometer; and combinations thereof.

Preferably, the biological sample obtained from the subject is selected from the group consisting of cell lines, histological slides, biopsies, paraffin-embedded tissue, bodily fluids, ejaculate, urine, blood, and combinations thereof.

Further embodiments provide an isolated treated nucleic acid derived from SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof, wherein the treatment is suitable to convert at least one unmethylated cytosine base of the genomic DNA sequence to uracil or another base that is detectably dissimilar to cytosine in terms of hybridization.

Additional embodiments provide a nucleic acid, comprising at least 16 contiguous nucleotides of a treated genomic DNA sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof, wherein the treatment is suitable to convert at least one unmethylated cytosine base of the genomic DNA sequence to uracil or another base that is detectably dissimilar to cytosine in terms of hybridization. Preferably, the contiguous base sequence comprises at least one CpG, TpG or CpA dinucleotide sequence. Preferbly, the treatment comprises use of a reagent selected from the group consisting of bisulfite, hydrogen sulfite, disulfite, and combinations thereof.

Yet additional embodiments provide an oligomer, comprising a sequence of at least 9 contiguous nucleotides that is complementary to, or hybridizes under stringent conditions to a

bisulfite-converted sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof. Preferably, the oligomer comprises at least one CpG , CpA or TpG dinucleotide sequence.

Also provided is a set of oligomers, comprising at least two oligonucleotides according, in each case, to those described above.

Preferred embodiments provide a novel use of a set of oligonucleotides as disclosed herein for at least one of: detection of; detection and differentiation between or among subclasses or stages of; diagnosis of; prognosis of; treatment of; monitoring of; and treatment and monitoring of prostate cell proliferative disorders.

Additional preferred aspects provide use of the disclosed inventive nucleic acids, the disclosed inventive oligomers, or a disclosed set of inventive oligonucleotides for detecting, or detecting and distinguishing between or among prostate cell proliferative disorders or stages thereof selected from the group consisting of: adjacent benign tissues; intermediate, T2, Gleason score 6 lymph node positive or negative tissue; high grade, T3, Gleason score 9 lymph node positive or negative tissue; prostatic adenocarcinoma; and metastatic tumors.

Alternate embodiments provide for use of a set of inventive oligomers as probes for determining at least one of a cytosine methylation state, and a single nucleotide polymorphism (SNP) of a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and sequences complementary thereto. Preferably, at least two inventive oligomers are used as primer oligonucleotides for the amplification of a DNA sequence of at least 16 contiguous nucleotides of a bisulfite-converted sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof.

Also disclosed and provided is the use of an inventive nucleic acid for determination of at least one of cytosine methylation status of a corresponding genomic DNA, or detection of a single nucleotide polymorphism (SNP).

Additional embodiments provide a method for manufacturing a nucleic acid array, comprising at least one of attachment of an inventive oligomer, or attachment of a set of such oligomers or nucleic acids, to a solid phase. Further embodiments provide an oligomer array

manufactured as described herein. Preferably, the oligomers are bound to a planar solid phase in the form of a lattice selected from the group consisting of linear or substantially linear lattice, hexagonal or substantially hexagonal lattice, rectangular or substantially rectangular lattice, and lattice combinations thereof. In preferred embodiments, the oligomer arrays are used for the analysis of prostate cell proliferative disorders. Preferably, the solid phase surface comprises a material selected from the group consisting of silicon, glass, polystyrene, aluminum, steel, iron, copper, nickel, silver, gold, and combinations thereof.

Yet further embodiments provide a kit useful for detecting, or for detecting and distinguishing between or among prostate cell proliferative disorders or stages thereof of a subject, comprising: at least one of a bisulfite reagent, and a methylation-sensitive restriction enzyme; and at least one nucleic acid molecule or peptide nucleic acid molecule comprising, in each case a contiguous sequence at least 9 nucleotides that is complementary to, or hybridizes under stringent conditions to a bisulfite-converted sequence derived from a sequence selected from the group consisting of SEQ ID NOS:1, 29, 31, 32, 34, 35, 37, 38, 40, 42, 43, 45, 47, 49, 51, and complements thereof. Preferably, the kit further comprises standard reagents for performing a methylation assay selected from the group consisting of MS-SNuPE, MSP, MethyLight, HeavyMethyl, COBRA, nucleic acid sequencing, and combinations thereof. Preferably, the above described methods comprise use of the kit according to claim 68.

Additional embodiments provide for use of: an inventive nucleic acid, an inventive oligomer, a set of inventive oligomers, a method of array manufacturing as described herein, an inventive array, and an inventive kit for the detection of, detection and differentiation between or among subclasses or stages of, diagnosis of, prognosis of, treatment of, monitoring of, or treatment and monitoring of prostate cell proliferative disorders.

Pharmaceutical Compositions and Therapeutic Uses

Pharmaceutical compositions of the invention can protein and protein-based agents of the claimed invention in a therapeutically effective amount. The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect

can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in advance. However, the effective amount for a given situation is determined by routine experimentation and is within the judgment of the clinician. For purposes of the present invention, an effective dose will generally be from about 0.01 mg/ kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the protein or polypeptide constructs in the individual to which it is administered. A non-limiting example of a pharmaceutical composition is a composition that either enhances or diminishes signaling mediated by a target receptor. Where such signaling promotes a disease-related process, modulation of the signaling would be the goal of the therapy.

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which can be administered without undue toxicity. Suitable carriers can be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Pharmaceutically acceptable carriers in therapeutic compositions can include liquids such as water, saline, glycerol and ethanol. Auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, can also be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier. Pharmaceutically acceptable salts can also be present in the pharmaceutical composition, *e.g.*, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically

acceptable excipients is available in *Remington's Pharmaceutical Sciences* (Mack Pub. Co., New Jersey, 1991).

Delivery Methods. Once formulated, the compositions of the invention can be administered directly to the subject or delivered ex vivo, to cells derived from the subject (*e.g.*, as in *ex vivo* gene therapy). Direct delivery of the compositions will generally be accomplished by parenteral injection, *e.g.*, subcutaneously, intraperitoneally, intravenously or intramuscularly, myocardial, intratumoral, peritumoral, or to the interstitial space of a tissue. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal applications, needles, and gene guns or hyposprays. Dosage treatment can be a single dose schedule or a multiple dose schedule.

Methods for the ex vivo delivery and reimplantation of transformed cells into a subject are known in the art and described in *e.g.*, International Publication No. WO 93/14778. Examples of cells useful in ex vivo applications include, for example, stem cells, particularly hematopoetic, lymph cells, macrophages, dendritic cells, or tumor cells. Generally, delivery of nucleic acids for both ex vivo and *in vitro* applications can be accomplished by, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, direct microinjection of the DNA into nuclei, and viral-mediated, such as adenovirus or alphavirus, all well known in the art.

In a preferred embodiment, disorders of proliferation, such as cancer, can be amenable to treatment by administration of a therapeutic agent based on the provided polynucleotide or corresponding polypeptide. The therapeutic agent can be administered in conjunction with one or more other agents including, but not limited to, receptor-specific antibodies and/or chemotherapeutic agents. Administered "in conjunction" includes administration at the same time, or within 1 day, 12 hours, 6 hours, one hour, or less than one hour, as the other therapeutic agent(s). The compositions may be mixed for co-administration, or may be administered separately by the same or different routes.

The dose and the means of administration of the inventive pharmaceutical compositions are determined based on the specific qualities of the therapeutic composition, the condition, age, and weight of the patient, the progression of the disease, and other relevant factors. For example, administration of polynucleotide therapeutic compositions agents of the invention includes local or

systemic administration, including injection, oral administration, particle gun or catheterized administration, and topical administration. The therapeutic polynucleotide composition can contain an expression construct comprising a promoter operably linked to a polynucleotide encoding, for example, about 80 to 419 (or about 350 to 419) contiguous amino acids of SEQ ID NO:2. Various methods can be used to administer the therapeutic composition directly to a specific site in the body. For example, a small metastatic lesion is located and the therapeutic composition injected several times in several different locations within the body of tumor. Alternatively, arteries which serve a tumor are identified, and the therapeutic composition injected into such an artery, in order to deliver the composition directly into the tumor. A tumor that has a necrotic center is aspirated and the composition injected directly into the now empty center of the tumor. X-ray imaging is used to assist in certain of the above delivery methods.

Protein-, or polypeptide-mediated targeted delivery of therapeutic agents to specific tissues can also be used. Receptor-mediated DNA delivery techniques are described in, for example, Findeis et al., *Trends Biotechnol.* (1993) *11*:202; Chiou et al., *Gene Therapeutics: Methods And Applications Of Direct Gene Transfer* (J.A. Wolff, ed.) (1994); Wu et al., *J. Biol. Chem.* (1988) *263*:621; Wu et al., *J. Biol. Chem.* (1994) *269*:542; Zenke et al., *Proc. Natl. Acad. Sci. (USA)* (1990) *87*:3655; Wu et al., *J. Biol. Chem.* (1991) *266*:338. Therapeutic compositions containing a polynucleotide are administered in a range of about 100 ng to about 200 mg of DNA for local administration in a gene therapy protocol. Concentration ranges of about 500 ng to about 50 mg, about 1 mg to about 2 mg, about 5 mg to about 500 mg, and about 20 mg to about 100 mg of DNA can also be used during a gene therapy protocol. Factors such as method of action (*e.g.*, for enhancing or inhibiting levels of the encoded gene product) and efficacy of transformation and expression are considerations which will affect the dosage required for ultimate efficacy of the subgenomic polynucleotides. Where greater expression is desired over a larger area of tissue, larger amounts of subgenomic polynucleotides or the same amounts readministered in a successive protocol of administrations, or several administrations to different adjacent or close tissue portions of, for example, a tumor site, may be required to effect a positive therapeutic outcome. In all cases, routine experimentation in clinical trials will determine specific ranges for optimal therapeutic effect.

Gene Therapy. The therapeutic polynucleotides and polypeptides of the present invention can be delivered using gene delivery vehicles. The gene delivery vehicle can be of viral or non-viral origin (see generally, Jolly, *Cancer Gene Therapy* (1994) *1*:51; Kimura, *Human Gene Therapy* (1994) *5*:845; Connelly, *Human Gene Therapy* (1995) *1*:185; and Kaplitt, *Nature Genetics* (1994) *6*:148). Expression of such coding sequences can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence can be either constitutive or regulated.

Viral-based vectors for delivery of a desired polynucleotide and expression in a desired cell are well known in the art. Exemplary viral-based vehicles include, but are not limited to, recombinant retroviruses (see, *e.g.*, WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; U.S. Patent No. 5, 219,740; WO 93/11230; WO 93/10218; U.S. Patent No. 4,777,127; GB Patent No. 2,200,651; EP 0 345 242; and WO 91/02805), alphavirus-based vectors (*e.g.*, Sindbis virus vectors, Semliki forest virus (ATCC VR-67; ATCC VR-1247), Ross River virus (ATCC VR-373; ATCC VR-1246) and Venezuelan equine encephalitis virus (ATCC VR-923; ATCC VR-1250; ATCC VR 1249; ATCC VR-532), and adeno-associated virus (AAV) vectors (see, *e.g.*, WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655). Administration of DNA linked to killed adenovirus as described in Curiel, *Hum. Gene Ther.* (1992) *3*:147 can also be employed.

Non-viral delivery vehicles and methods can also be employed, including, but not limited to, polycationic condensed DNA linked or unlinked to killed adenovirus alone (see, *e.g.*, Curiel, *Hum. Gene Ther.* (1992) *3*:147); ligand-linked DNA (see, *e.g.*, Wu, *J. Biol. Chem. 264*:16985 (1989)); eukaryotic cell delivery vehicles cells (see, *e.g.*, U.S. Patent No. 5,814,482; WO 95/07994; WO 96/17072; WO 95/30763; and WO 97/42338) and nucleic charge neutralization or fusion with cell membranes. Naked DNA can also be employed. Exemplary naked DNA introduction methods are described in WO 90/11092 and U.S. Patent No. 5,580,859. Liposomes that can act as gene delivery vehicles are described in U.S. Patent No. 5,422,120; WO 95/13796; WO 94/23697; WO 91/14445; and EP 0524968. Additional approaches are described in Philip, *Mol. Cell Biol. 14*:2411 (1994), and in Woffendin, *Proc. Natl. Acad. Sci.* (1994) *91*:11581-11585.

Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin et al., *Proc. Natl. Acad. Sci. USA 91*(24):11581 (1994). Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials or use of ionizing radiation (see, *e.g.*, U.S. Patent No. 5,206,152 and WO 92/11033). Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun (see, *e.g.*, U.S. Patent No. 5,149,655); use of ionizing radiation for activating transferred gene (see, *e.g.*, U.S. Patent No. 5,206,152 and WO 92/11033).

The present invention will now be illustrated by reference to the following examples which set forth particularly advantageous embodiments. However, it should be noted that these embodiments are illustrative and are not to be construed as restricting the invention in any way.

EXAMPLE 1

(A set of genes was identified that characterize prostate cancer and benign prostatic tissues)

Materials and methods

Prostate tissues. Prostate cancer tissue specimens were obtained from patients who had undergone radical prostatectomy for prostate cancer at Mayo Clinic. The Institutional Review Board of Mayo Foundation approved collection of tissues, and their use for this study. None of the patients included in this study had received preoperative hormonal therapy, chemotherapy, or radiotherapy. Harvested tissues were embedded in OCT and frozen at –80°C until use. A hematoxylin and eosin stained section was prepared to insure that tumor was present in the tissue used for the analyses. Out of 340 tissues available in our tissue bank, we selected tissues that had more than 80% of the neoplastic cells by histological examination. In order to examine differential gene expression in intermediate (Gleason score 6), high grade (Gleason score 9) prostatic adenocarcinoma and metastatic tumors, we studied 11 primary stage T2 Gleason score 6 cancers (six with positive regional lymph nodes and five with negative lymph nodes), 12 primary stage T3 Gleason score 9 cancers (six with positive regional lymph nodes, six with negative lymph nodes), and five metastatic tumors.

TABLE 1 shows Gleason grade, age, pre-operative serum prostate-specific antigen levels and staging of all patients from whom prostate tissues were obtained for this study. Twelve separately

collected prostatic tissue samples matched with the cancer tissues (obtained from the same patients) were used as normal controls.

TABLE 1. Prostate tissue samples with preoperative PSA values at diagnosis, Gleason histological scores, and metastasis status of the tissues.

Gleason grade/Lymph node	Sample ID	Age	Preop PSA (ng/ml)	TNM (97)	Metastatic site
6/Negative	6N 1	55	9.4	T2b,N0-	
	6N 2	50	7.5	T2b,N0-	
	6N 3	57	10.3	T2b,N0-	
	6N 4	67	16.7	T2b,N0-	
	6N 5	68	8.1	T2a,N0-	
6/Positive	6P 1	71	17.1	T2b,N1+	
	6P 2	61	5.2	T2b,N0+	
	6P 3	71	41.0	T2b,N0+	
	6P 4	65	7.0	T2a,N0+	
	6P 5	51	14.3	T2b,N0+	
	6P 6	66	23.5	T2b,N0+	
9/Negative	9N 1	67	21.6	T3a,N0-	
	9N 2	65	29.4	T3b,N0-	
	9N 3	65	24.9	T3b,N0-	
	9N 4	54	50.0	T3b,N0-	
	9N 5	59	25.8	T3b,N0-	
	9N 6	71	6.1	T3b,N0-	
9/Positive	9P 1	66	4.5	T3a,N0+	
	9P 2	65	6.69	T3b,N0+	
	9P 3	76	7.6	T3b,N1+	
	9P 4	71	467.0	T3b,N0+	
	9P 5	69	5.6	T3b,N0+	
	9P 6	66	2.9	T3b,N1-	
Metastatic	Met 1	62	0.15		Liver
	Met 2	72	97.3		Peritoneum
	Met 3	49	0.15		Lymph node
	Met 4	60	18.4		Lymph node
	Met 5	68	8.9		Lung

Isolation of RNA and gene expression profiling. Thirty prostate tissue sections of 15-μm thicknesses were cut with a cryostat and used for RNA isolation. Total RNA was extracted from frozen tissue sections with Trizol® reagent (Life Technologies, Inc., Carlsbad, CA). DNA was removed by treatment of the samples with DNase I using DNA-free™ kit (Ambion, Austin, TX) and further RNA cleanup was performed using RNeasy Mini kit (Qiagen, Valencia, CA) according to the manufacturer's protocols. RNA quality was monitored by agarose gel electrophoresis and also on

Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). High-density oligonucleotide s HG-U95Av2 containing 12,625 sequences of human genes and ESTs (Affymetrix, Santa Clara, CA) were used in this study. Complementary RNA was prepared, labeled and hybridized to oligonucleotide arrays as described previously (Giordano et al., *Am. J. Pathol.* 159: 1231-1238, 2001). The arrays were scanned with gene array scanner (Agilent Technologies, Palo Alto, CA). All arrays were scaled to a target intensity of 1500. Raw data was collected and analyzed by using Affymetrix Suite 5.0 version.

Quantitative Real-Time RT-PCR. To confirm the differential expression of genes from data, four down-regulated genes, ZNF185, PSP94, BPAG1 and TGM4 and two up-regulated genes Erg-2 and RhoGDI-β were selected for validation by Taqman real-time RT-PCR in a total of 44 tissues, including 36 samples used for s with an additional 4 primary tumors and 4 adjacent benign tissues. One (1) μg of the total RNA was used for first-strand cDNA synthesis. The PCR mix contained 1X reaction buffer (10 mM Tris, 50 mM KCl, pH 8.3), $MgCl_2$ (5 mM), PCR nucleotide mix (1 mM), random primers (0.08 A260 units), RNase inhibitor (50 units), AMV reverse transcriptase (20 units) in a final volume of 20 μl.

For real-time PCR one μl of the cDNA was used in the PCR reactions. Taqman real-time primers and probes were designed using the software Primer Express™ version 1.5 (PE Applied Biosystems, Foster City, CA) and synthesized at Integrated DNA Technologies (Coralville, IA). The sequences of the primers and probes for each gene are provided in TABLE 2 and FIGURE 2(a).

TABLE 2. Sequences of the primers and probes.

Gene		Primers and Probe	Amplicon bp	SEQ ID NO.
ZNF185	FP	TGGATGAAAGGCAAGGTAAAGAG	84	3
	RP	TTCTAAAACTCCCTTAAAGGCAGACT		4
	Probe	CCAAGATAGGCTGGCTTCCCCCG		5
PSP94	FP	AGTGAATGGATAATCTAGTGTGCTTCTAGT	100	6
	RP	GCATGGCTACACAATCATTGACTAT		7
	Probe	CCCAGGCCAGGCCTCATTCTCCT		8
BPAG1	FP	TCGCTGAAAGAGCACGTCAT	94	9
	RP	AGCAATCTAAAACACTGCAGCTTG		10
	Probe	AATCAAAGAGAAAGATATAAATTCGTTCCCACAGCC		11
Erg-2	FP	TCCTGTCGGACAGCTCCAAC	75	12
	RP	CGGGATCCGTCATCTTGA		13
	Probe	TGCATCACCTGGGAAGGCACCAAC		14

Probes were labeled at 5' end with the reporter dye 6-carboxyfluorescein (6'-FAM) and at 3' end with a Black Hole Quencher (BHQ). Probes were purified by reverse phase HPLC and primers

were PAGE purified. All PCR reactions were carried out in Taqman Universal PCR master mix (PE Applied Biosytems) with 300 nM of each primer and 200 nM of probe in a final volume of 50 µl. Thermal cycling conditions were as follows: 2 min at 50°C, with denaturation at 95°C for 10 min, 40 cycles of 15 sec at 95°C (melting) and 1 min at 60°C (annealing and elongation). The reactions were performed in an ABI Prism® 7700 Sequence Detection System (PE Applied Biosystems). To evaluate the validity and sensitivity of real-time quantitative PCR, serial dilutions of the oligonucleotide amplicon of the gene in a range of 1 to 1 x 10^9 copies were used as corresponding standard. Standard curves were generated using the C_t values determined in the real-time PCR to permit gene quantification using the supplied software according to the manufacturer's instructions. In addition, a standard curve was generated for the housekeeping gene, glyceraldehyde-3-phosphate-dehydrogenase (Applied Biosystems, part number 402869) to enable normalization of each gene. Data were expressed as relative copy number of transcripts after normalization.

Cell Lines and 5-Aza-CdR Treatment. The human prostate cancer cell lines LNCaP, PC3 (American Type Culture Collection, Rockville, MD, USA) and LAPC4 (a gift from Dr. Charles L. Sawyers, University of California, Los Angeles, CA) were grown in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 5% fetal bovine serum (FBS) at 37°C and 5% CO_2 until reaching approximately 50-70% confluence. Cells were then treated with 5% FBS RPMI 1640 containing 6µM 5-aza-2'-deoxycytidine (5-Aza-CdR) (Sigma Chemicals Co., St. Louis, MO) for 6 days, with medium changes on day 1, 3, and 5. Total RNA was isolated from the cell lines and the expression of the ZNF185 was analyzed by Taqman real-time PCR as described above. The housekeeping gene GAPDH was used as an internal control to enable normalization.

DNA isolation and Bisulfite modification. Genomic DNA was obtained from metastatic, primary, matched benign prostatic tissues and the above mentioned prostate cancer cell lines treated with 5-Aza-CdR, using Wizard® genomic DNA purification kit according to the manufacturer's protocol (Promega, Madison, WI). Genomic DNA (100 ng) was modified by sodium bisulfite treatment by converting unmethylated, but not methylated, cytosines to uracil as described previously (Herman et al., *Proc. Natl. Acad. Sci. USA* 93:9821-9826, 1996). DNA samples were then purified using the spin columns (Qiagen), and eluted in 50 µl of distilled water. Modification was completed by treatment with NaOH (0.3 M final concentration) for 5 min at room temperature, followed by ethanol precipitation. DNA was re-suspended in water and used for PCR amplification.

Methylation Specific PCR (MSP). DNA methylation patterns within the gene were determined by chemical modification of unmethylated cytosine to uracil and subsequent PCR as described previously (Esteller et al., *Cancer Res.* 61:3225-3229, 2001), using primers specific for

either methylated or the modified unmethylated sequences. The primers used for MSP were shown in TABLE 3 and FIGURE 3(b).

TABLE 3. Primers used for MSP analysis.

Primer set			Size bp	Genomic position	SEQ ID NO.
1 W	FP	GCGCAGTTCCGGGTGTCTGTC	197	210	15
	RP	GCGGGGAGGACCAGCGTTAG			16
1 M	FP	GCGTAGTTTCGGGTGTTTG	197	210	17
	RP	ACGAAAAAAACCAACGTTAACTA			18
1 U	FP	GTGTAGTTTTGGGTGTTTGTTAGG	196	210	19
	RP	CAAAAAAAACCAACATTAACTATTCTC			20
2 W	FP	CCTGGGACTCCGTCAGACTGG	146	335	21
	RP	GACAGACACCCGGAACTGCG			22
2 M	FP	TTGGGATTTCGTTAGATTGG	145	335	23
	RP	AACAAACACCCGAAACTACG			24
2 U	FP	TGGGATTTTGTTAGATTGGAAAGG	146	333	25
	RP	CTAACAAACACCCAAAACTACACCA			26

Two sets of primers were designed corresponding to the genomic positions around 210 and 335. Genomic position indicates the location of the 5' nucleotide of the sense primer in relation to the major transcriptional start site defined in the Genbank accession number (Y09538). The PCR mixture contained 1X PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3 with 0.01% w/v gelatin), dNTPs (0.2 mM each), primers (500 μM) and bisulfite modified or unmodified DNA (100 ng) in a final volume of 25 μl. Reactions were hot-started at 95°C for 10 min with the addition of 1.25 units of AmpliTaq Gold™ DNA polymerase (PerkinElmer). Amplifications were carried out in GeneAmp PCR systems 9700 (Applied Biosystems) for 35 cycles (30 sec at 95°C, 30 sec at 55°C and 30 sec at 72°C), followed by a final 7 min extension at 72°C. Appropriate negative and positive controls were included in each PCR reaction. One (1) μl of the PCR product was directly loaded onto DNA 500 lab chip and analyzed on Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA).

RESULTS

Gene expression profiles of 28 prostate cancer tissues were monitored using oligonucleotide s. A gene-by-gene analysis of the difference in mean log expression between the two groups was performed to identify genes differentially expressed between cancer and benign tissues. Genes were ranked according to inter-sample variability (SD), and 1850 genes with the most variable expression

across all of the samples were median-centered and normalized with respect to other genes in the samples and corresponding genes in the other samples. Genes and samples were subjected to hierarchical clustering essentially as described previously (Eisen et al., *Proc. Natl. Acad. Sci. USA* 95:14863-14868, 1998). Differential expression of genes in benign and malignant prostate tissues was estimated using an algorithm (Giordano et al., *Am. J. Pathol.* 159:1231-1238, 2001) based on equally weighted contributions from the difference of hybridization intensities (μTumor-μNormal) or (μNormal-μTumor), the quotient of hybridization intensities (μTumor/μNormal) or (μNormal/μTumor), and the result of an unpaired *t*-test between expression levels in tumor and normal tissues. The selection criteria was narrowed to genes that showed a fold change of >2.35 between normal and cancer samples and a $p<0.001$ by student's t-test. A cluster of 25 up-regulated and 25 down-regulated genes, which discriminated between normal and cancer tissues was identified (Fig. 1).

Among the 25 down-regulated genes identified (Fig.1), PSP94, BPAG1, WFDC2, KRT5, KRT15, TAGLN, ZFP 36 and the genes encoding LIM domain proteins FLH1, FLH2, ENIGMA are consistent with the expression profiles of previous studies (Dhanasekaran et al., *Nature* 412:822-826, 2001; Ernst et al., *Am. J. Pathol.* 160:2169-2180, 2002; LaTulippe et al., Cancer Res. 62:4499-4506, 2002; Luo et al., *Mol. Carcinog.* 33:25-35, 2002; Shields et al., *J. Biol. Chem.* 277:9790-9799, 2002). Up-regulation of hepsin, AMACR, STEAP, FOLH1, RAP2A and the unknown gene DKFZP564B167 are consistent with the previously published data of analysis (Dhanasekaran et al., *supra*; Luo et al., *Cancer Res.* 61:4683-4688, 2001; Magee et al., *Cancer Res.* 61:5692-5696, 2001; Welsh et al., *Cancer Res.* 61:5974-5978, 2001; Rubin et al., *Journal of the American Medical Assn.* 287:1662-1670, 2002; Ernst et al., *supra*; Luo et al., supra; Rhodes et al., *Cancer Res.* 62:4427-4433, 2002; Stamey et al., *J. Urol.* 166:2171-2177, 2001). In addition, the present data also confirms up-regulation of the cell cycle regulated genes CCNB1, CCNB2, MAD2L1, DEEPEST, BUB1B, cell adhesion regulator MACMARCKS, and unclassified genes KIAA0186 and KIAA0906 (Welsh et al., *supra;* Ernst et al., *supra;* LaTulippe et al., *supra;* Stamey et al., *supra*).

PSP94, ZNF185, BPAG1, and TGM4 were selected from the 25 down-regulated genes and Erg-2 and RhoGDI-β from the 25 up-regulated genes for further validation by Taqman quantitative PCR. These genes were selected because of their moderate to high level expression in prostate cancer. In addition, their potential functions, as mentioned below, are relevant to prostate cancer biology. Furthermore, except for PSP94, their role in prostate cancer biology has not been previously described. PSP94 has been shown to be down-regulated in prostate cancer (Sakai et al.,

Prostate 38:278-284, 1999) and is the most down-regulated gene in the instant data.

To validate the expression profiles, Taqman quantitative PCR was performed in duplicate for each sample. The standard curve slope values for all the genes ranged between –3.58 and –3.20, corresponding to PCR efficiency of above 0.9. The Kruskal-Wallis global test was done with the real time quantitative analysis for all the genes. A significant decrease in the expression of ZNF185, BPAG1 and PSP94 mRNA levels was observed in metastatic *versus* organ confined and localized tumors compared to benign tissues [$p<0.0001$] (Fig. 2b). Moreover, the Wilcoxon test was used to compare each tissue type to the adjacent benign tissues. ZNF185, BPAG1 and PSP94 showed p-values less than 0.0019 in each group compared to benign tissues.

PSP94 is a highly prostate specific gene encoding a major prostate secretory protein. Earlier studies reported that both the secretion and synthesis of PSP94 were reduced in prostate cancer tissues (Sakai et al., *supra*). PSP94 is involved in inhibition of tumor growth by apoptosis (Garde et al., *Prostate* 38:118-125, 1999) and the down-regulation in prostate tumor tissues may be the survival mechanism for cancer cells. The instant experiments indicate that PSP94 palys a role in prostate cancer progression.

BPAG1 is a 230-kDa hemi-desmosomal component involved in adherence of epithelial cells to the basement membrane. Previous studies have shown a loss of BPAG1 in invasive breast cancer cells (Bergstraesser et al., *Am. J. Pathol.* 147:1823-1839, 1995). The down-regulation of BPAG1 in our study (>14 fold in metastatic tissues) provides an indicator of an invasive phenotype and predicts the potential of invasive cells to metastasize (Herold-Mende et al., *Cell Tissue Res.* 306:399-408, 2001).

Erg-2 is a proto-oncogene known to play an important role in the development of cancer (Simpson et al., *Oncogene* 14:2149-2157, 1997). Erg-2 expression levels were herein observed to increased in 16 (50%) out of 32 cancer tissues when stringently compared to the highest level of Erg-2 in 12 adjacent benign tissues. The increase in mRNA levels of Erg-2 in at least half of the cancer tissues examined indicates a role of Erg-2 in prostate cancer.

Furthermore, TGM4 is a prostate tissue specific transglutaminase (type IV) that has been implicated in apoptosis and cell growth (Antonyak et al., *J. Biol. Chem.* 278:15859-15866, 2003). RhoGDI- β may be involved in cellular transformation (Lozano et al., *Bioessays* 25:452-463, 2003). The present Taqman PCR study shows that TGM4 and RhoGDI- β levels were not changed significantly in most of the prostate cancer tissues (data not shown).

ZNF185 is a novel LIM domain gene (Heiss et al., *Genomics* 43:329-338, 1997), and, according to the present invention, plays a role in prostate cancer development and progression.

Particular LIM domain proteins have been shown to play an important role in regulation of cellular proliferation and differentiation (Bach, I., *Mech Dev.* 91:5-17, 2000; McLoughlin, et al., *J. Biol. Chem.* 277:37045-37053, 2002; Mousses et al., *Cancer Res.* 62: 1256-1260, 2002; Yamada et al., *Oncogene*, 21:1309-1315, 2002; Robert et al., *Nat. Genet.* 33:61-65, 2003). ZNF185 is located on chromosome Xq28, a chromosomal region of interest as a result of the more than 20 hereditary diseases mapped to this region. The ZNF185 LIM is a cysteine-rich motif that coordinately binds two zinc atoms and mediates protein-protein interactions. Heiss et al. (Heiss et al., *supra*) cloned a full-length ZNF185 cDNA and showed that the transcript is expressed in a very limited number of human tissues with most abundant expression in the prostate.

Significantly, the present invention is the first identification of a correlation of ZNF185 regulation and cancer. Specifically, there was a significant down-regulation in the expression of ZNF185 gene in all prostate cancer tissues compared to benign prostatic tissues (Fig. 1 and 2b). The decrease in ZNF185 expression in prostate tumors indicated that ZNF185 plays an important role in the development and progression of prostate cancer.

To study the transcriptional silencing of ZNF185 in prostate cancer, LAPC4, LNCaP and PC3 prostate cancer cell lines were treated with 5-Aza-CdR an inhibitor of DNA methyl transferase DNMT1 (Robert et al., *supra*). Treatment with 5-Aza-CdR showed approximately a 2.0-fold increase in mRNA levels of ZNF185 (Fig 3a, indicating that the gene might be partially silenced by methylation. To confirm the transcriptional inactivation, MSP was carried out to assess the methylation status of cytosine residues in the 5' CpG dinucleotides of genomic DNA in prostate tumors, adjacent benign tissues and in prostate cell lines with or without treatment with 5-Aza-CdR. Cytosine methylations within CpG dinucleotides were observed in the prostate cancer tissues and cell lines with two sets of primers used for PCR (Fig 3c). A reduction of the methylated band and increase of the unmethylated band in cell lines with 5-Aza-CdR treatment is consistent with the restoration of ZNF185 mRNA levels after demethylation. (Fig 3a).

In most of tissues samples, DNA not treated with bisulfite (unmodified) failed to amplify with either set of methylated or unmethylated specific primers but readily amplified with primers specific for the sequence before modification, suggesting an almost complete bisulfite reaction. Methylation of ZNF185 was accompanied by amplification of the unmethylated reaction as well. The presence of the unmethylated ZNF185 DNA could indicate the presence of normal tissues in these non-microdissected samples. However, heterogeneity in the patterns of methylation in the tumor itself might also be present. Fisher's unordered test for methylation difference in metastatic, confined tumors and benign tissues was highly significant ($p<0.0003$).

The incidence of methylation in cancer tissues is shown in Fig. 3(d). Methylation status and down-regulation in the mRNA expression is correlated with higher tumor grade and metastasis.

These results indicate that methylation of CpG dinucleotides may be the major factor causing transcriptional inactivation of ZNF185 and repressing its expression in the prostate cancer tissues.

In summary, mRNA expression analysis with oligonucleotide s identified a set of genes that characterize prostate cancer and benign prostatic tissues. A decrease in the expression of genes PSP94, BPAG1 and ZNF185 highly correlates with prostate cancer progression. Increase of Erg-2 levels also indicates its role in development of prostate cancer.

Significantly, this is the first study to identify inactivation of the LIM domain gene ZNF185 in patients with prostate cancer and in prostate cancer cell lines. The present invention identifies this gene as a marker of prostate cancer aggressiveness. According to the present invention, transcriptional silencing of PSP94 and BPAG1 additionally serves as prognostic markers for prostate cancer progression, and as potential therapeutic targets for prostate cancer.

TABLE 1. Prostate tissue samples with preoperative PSA values at diagnosis, Gleason histological scores, and metastasis status of the tissues.

Gleason grade/Lymph node	Sample ID	Age	Preop PSA (ng/ml)	TNM (97)	Metastatic site
6/Negative	6N 1	55	9.4	T2b,N0-	
	6N 2	50	7.5	T2b,N0-	
	6N 3	57	10.3	T2b,N0-	
	6N 4	67	16.7	T2b,N0-	
	6N 5	68	8.1	T2a,N0-	
6/Positive	6P 1	71	17.1	T2b,N1+	
	6P 2	61	5.2	T2b,N0+	
	6P 3	71	41.0	T2b,N0+	
	6P 4	65	7.0	T2a,N0+	
	6P 5	51	14.3	T2b,N0+	
	6P 6	66	23.5	T2b,N0+	
9/Negative	9N 1	67	21.6	T3a,N0-	
	9N 2	65	29.4	T3b,N0-	
	9N 3	65	24.9	T3b,N0-	
	9N 4	54	50.0	T3b,N0-	
	9N 5	59	25.8	T3b,N0-	
	9N 6	71	6.1	T3b,N0-	
9/Positive	9P 1	66	4.5	T3a,N0+	
	9P 2	65	6.69	T3b,N0+	
	9P 3	76	7.6	T3b,N1+	
	9P 4	71	467.0	T3b,N0+	
	9P 5	69	5.6	T3b,N0+	

Gleason grade/Lymph node	Sample ID	Age	Preop PSA (ng/ml)	TNM (97)	Metastatic site
	9P 6	66	2.9	T3b,N1-	
Metastatic	Met 1	62	0.15		Liver
	Met 2	72	97.3		Peritoneum
	Met 3	49	0.15		Lymph node
	Met 4	60	18.4		Lymph node
	Met 5	68	8.9		Lung

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EXAMPLE II

(624 genes were identified by expression profiling as having differential expression patterns in metastatic and confined prostate tumors relative to benign tissues, eleven (11) of these genes were further validated as diagnostic/prognostic markers by quantitative real time PCR validation, and 5 genes were shown to be silenced, at least in part, by DNA methylation)

In this Example, the expression of genes in benign and untreated human prostate cancer tissues was profiled using oliginucleotide s (Affymetrix U133A and U133B chips). Six hundred-twenty four (624) genes were shown by the analysis to have distinct expression patterns in metastatic and confined tumors (Gleason score 6 and 9, relative to benign tissues. A total of eleven (11) of these differentially expressed genes were selected and further validation by Taqman quantitative real time PCR to confirm the differential expression of genes according to the data.

Materials and Methods:

Prostate Tissue. Prostate cancer tissue specimens were obtained from patients who had undergone radical prostatectomy for prostate cancer at Mayo Clinic as described earlier (Vanaja et al., *Cancer Res.* 63:3877-3822, 2003).

TABLE 1 (herein below) shows Gleason grade, age, pre-operative serum prostate-specific antigen (PSA) levels at diagnosis, and staging (Gleason histological scores) of all patients from whom prostate tissues were obtained for this study. A total of 40 prostate tissues were used to study the gene expression profiling.

Isolation of RNA and Gene expression profiling. Thirty prostate tissue sections of 15-μm thicknesses were cut with a cryostat and used for RNA isolation. Total RNA was extracted from frozen tissue sections with Trizol® reagent (Life Technologies, Inc., Carlsbad, CA). High-density oligonucleotide s, U133A and U133B, containing 44792 sequences of human genes and ESTs

(Affymetrix, Santa Clara, CA) were used in this study. Complementary RNA was prepared, labeled and hybridized to oligonucleotide arrays as described previously (Vanaja et al., *supra*).

The expression profiles were generated from 5 metastatic prostate tissues, and 27 confined tumors, including fifteen (15) Gleason score-9 (high grade) and twelve (12) Gleason score-6 (intermediate grade) tumors. Additionally, eight (8) adjacent benign prostatic tissues were also studied. Six hundred forty-two (642) genes with distinct (differential) expression patterns in prostate cancer compared with benign prostatic tissues were identified (*see* Table 2 herein below).

TABLE 2 shows the differential expression (relative to benign tissue) of 624 significantly regulated genes in 40 prostate tissue samples. The expression is computed as the average of the probes within each probe set of a gene in the chips. The 624 genes were 'extracted' from the metastatic vs. benign tissues with significant p-value < 0.01. The genes from the combined set of probes (U133A and U133B) were ranked by the ABS (t-statistic). Genes were selected for further study based on a t-statistics cutoff of 2 or above 2. A negative t-statistic value indicates a decrease in, and positive indicates an increase in the expression of genes in cancer tissues. The fold-change in the expression of genes in Metastatic, Gleason grade 9 and Gleason grade 6 as compared to adjacent benign tissues are shown at the right.

Quantitative Real-Time Reverse Transcriptase-PCR. Seven down-regulated genes and four up-regulated genes were selected for validation by Taqman real-time RT-PCR to confirm the micorarray-based differential expression of these genes. One (1) µl of the cDNA was used in the PCR reactions. Taqman real-time primers and probes were obtained from Applied Biosystems (Foster City, CA) for all genes, except that the primers and probe for FABP5 were designed by the present inventors and custom synthesized. The sequence of the forward and reverse primers used for FABP5 were as follows:

forward primer: GGAGTGGGATGGGAAGGAAAG (SEQ ID NO:27);

reverse primer: CACTCCACCACTAATTTCCCATCTT (SEQ ID NO:28);

reporter 1 Dye: FAM;

reporter 1 quencher: NFQ.

All probes were labeled at the 5' end with the reporter dye 6-carboxyfluorescein (6'-FAM) and at 3' end with a nonfluorescent quencher NFQ. All PCR reactions were carried out in TaqMan® Universal PCR master mix (PE Applied Biosystems) with 900 nM of each primer and 250 nM of probe in a final volume of 50 µl. Thermal cycling conditions were as follows: 2 min at 50°C, with denaturation at 95°C for 10 min, 40 cycles of 15 s at 95°C (melting) and 1 min at 60°C (annealing and elongation). The reactions were performed in an ABI Prism® 7700 Sequence

Detection System (PE Applied Biosystems). Standard curves were generated for the housekeeping gene, glyceraldehyde-3-phosphate-dehydrogenase (Applied Biosystems, part number 402869) to enable normalization of each gene. Data were expressed as relative fold changes in the mRNA expression by benign tissues after normalization with GAPDH levels (see FIGURE 1 and TABLE 4).

TABLE 4. Text corresponding to FIGURE 1.

		Expression ratio to mean ABT				
Gene	UniGene	Met	9P	9N	6P	6N
TGM4	Hs.2387	32.70+21.83	17.46+21.27	12.40+17.11	5.23+3.92	23.47+19.76
ZFP36	Hs. 343586	4.68+3.55	1.98+1.34	2.03+1.94	1.88+1.53	2.05+1.83
RIS1	Hs.35861	4.07+4.35	2.29+3.19	2.85+3.95	2.39+6.51	1.51+2.08
EFS2	Hs.24587	7.37+1.91	3.68+0.75	2.03+0.38	1.82+0.44	2.81+0.70
FLH2	Hs.8302	3.18+1.26	1.79+1.20	1.82+1.72	2.88+2.71	2.38+2.31
FOXF1	Hs.155591	8.43+4.01	2.34+1.42	2.19+0.87	3.19+2.26	2.88+2.18
ENIGMA	Hs.102948	3.73+2.16	2.21+1.10	1.42+0.92	3.38+2.43	3.28+3.05
FHL1	Hs.239069	5.25+2.69	1.94+0.87	1.82+0.97	3.40+1.71	2.78+1.65
PCP4	Hs.80296	32.45+12.40	3.06+1.64	2.36+1.51	5.22+4.09	3.38+14.14
CNN1	Hs.21223	52.53+27.61	4.59+1.95	3.12+1.38	5.17+3.60	4.09+2.72
TAGLN	Hs.75777	9.07+3.52	2.46+0.68	1.46+0.84	2.56+1.36	2.55+1.87
GSTM1	Hs.301961	4.10+0.87	3.58+1.83	2.07+0.69	2.92+0.91	3.39+2.66
CSRP1	Hs.108080	7.89+3.31	3.85+1.80	2.74+0.90	3.15+1.28	3.48+1.80
ZNF185	Hs.16622	11.17+4.38	3.21+1.30	3.89+0.82	3.67+1.61	2.63+0.56
TRIM29	Hs.82237	6.26+2.82	3.53+1.98	2.79+2.36	3.84+3.21	2.87+1.86
KRT5	Hs.195850	63.86+80.24	6.67+3.12	5.24+2.81	4.76+2.44	4.76+4.10
BPAG1	Hs.198689	14.04+6.03	3.75+1.74	5.03+4.19	4.14+2.10	7.76+5.82
PLP1	Hs.1787	5.09+3.00	2.54+1.74	2.08+2.45	3.01+2.75	3.21+2.53
PSP94	Hs.183752	129.56+59.19	3.18+2.12	4.14+1.34	2.36+1.64	2.88+1.84
LOC113146	Hs.57548	9.20+7.49	3.27+1.41	1.14+0.73	4.34+1.48	3.29+1.63
WFDC2	Hs.2719	11.21+3.80	2.71+0.62	4.35+2.78	5.24+2.49	3.14+2.64
NEFH	Hs.198760	14.89+7.11	17.22+14.17	4.59+1.85	2.95+1.74	1.69+0.75
KRT15	Hs.80342	90.71+213.95	6.38+5.01	5.24+5.63	4.68+5.26	4.45+4.94
GAGEC1	Hs.95420	63.35+27.88	2.58+0.84	2.39+0.69	1.59+0.48	2.58+0.85
ACPP	Hs.1852	7.22+2.24	2.23+1.07	3.87+3.07	1.00+4.90	1.24+2.06
TMSNB	Hs.56145	3.13+7.91	2.97+5.26	2.55+5.63	4.64+8.58	3.85+2.95
AMACR	Hs.128749	570+12.34	3.66+9.49	1.72+2.22	0.65+0.32	1.01+0.57
HPN	Hs.823	2.99+3.64	3.19+7.31	2.80+2.96	3.93+2.46	4.19+1.96
DKFZP564B167	Hs.76285	2.50+3.43	2.22+5.19	1.62+1.09	3.04+2.72	2.46+2.12
STEAP	Hs.61635	2.35+6.70	2.65+4.94	2.37+2.59	2.64+1.49	1.88+0.78
FOLH1	Hs.1915	3.76+10.12	2.61+3.17	1.90+2.11	2.14+1.89	3.01+2.15
CADPS	Hs.151301	10.26+11.52	2.30+2.56	1.04+0.58	0.25+0.19	1.13+0.40
LOC90355	Hs.25925	3.87+2.24	2.87+2.07	0.77+1.18	1.91+2.13	2.21+1.99
ERG2	Hs.45514	20.06+27.86	8.36+9.15	4.88+5.49	5.06+7.52	19.69+12.87
MACMARCKS	Hs.75061	3.08+12.35	2.62+4.51	2.45+7.68	2.95+4.70	4.02+2.80
GPC3	Hs.119651	4.79+1.19	5.73+8.89	1.79+1.88	2.01+2.39	1.34+1.20
KIAA9101	Hs.81892	5.51+5.53	5.43+5.69	2.08+0.91	2.76+0.95	2.90+0.49
TK1	Hs.105097	6.61+4.25	3.55+2.14	2.45+1.30	2.18+1.59	1.95+1.06
MAD2L1	Hs.79078	3.44+10.86	4.18+12.06	5.26+6.36	4.85+5.57	4.73+7.76
KIAA0906	Hs.56966	4.60+13.94	4.13+6.82	2.24+3.95	3.19+4.28	5.33+4.46
CCNB2	Hs.194698	5.94+7.85	4.05+11.25	2.10+2.52	2.47+3.83	2.83+2.65

Fold decrease

Fold increase

		Expression ratio to mean ABT				
Gene	UniGene	Met	9P	9N	6P	6N
BUB18	Hs.103834	2.37+5.29	2.99+5.24	2.13+1.64	1.60+2.19	2.08+1.36
CCNB1	Hs.23960	4.31+2.88	3.07+6.25	2.12+2.07	2.24+0.82	2.13+1.05
DEEPEST	Hs.16244	5.12+1.55	5.71+7.67	2.23+1.37	1.62+1.52	2.11+1.49
KIAA0186	Hs.36232	5.32+3.32	2.91+2.60	1.43+0.52	2.42+1.13	1.16+0.98
TNRC9	Hs.110826	6.76+6.11	8.22+6.60	2.16+1.14	6.09+2.54	8.03+2.95
RAP2A	Hs.355373	3.87+9.42	1.24+1.51	0.81+0.68	0.83+1.21	1.44+0.67
F2R	Hs.128087	5.65+11.11	10.49+13.61	8.82+13.08	17.51+15.26	21.01+15.34
ICAP-1alpha	Hs.356320	27.64+88.62	5.57+25.47	1.55+2.45	0.96+0.46	0.93+0.65
ARHGDIB	Hs.83656	4.04+1.27	3.02+3.17	1.59+0.79	2.09+0.69	2.19+0.53

Cell Lines and 5-Aza-CdR Treatment. The human prostate cancer cell lines LNCaP, PC3 (American Type Culture Collection, Rockville, MD, USA) and LAPC4 (a gift from Dr. Charles L. Sawyers, University of California, Los Angeles, CA) were grown in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 5% fetal bovine serum (FBS) at 37°C and 5% CO_2 until reaching approximately 50-70% confluence. Cells were then treated with 5% FBS RPMI 1640 containing 6μM 5-Aza-CdR (Sigma Chemicals Co., St. Louis, MO) for 6 days, with medium changes on day 1, 3, and 5. Total RNA was isolated from the cell lines and the expression of the genes was analyzed by TaqMan® real-time PCR as described above. Data were expressed as relative fold change in the mRNA expression by untreated controls (see FIGURE 2).

Results:

In the study of EXAMPLE I herein, fifty (50) genes were identified and disclosed that are significantly altered in prostate cancer tissues. In this EXAMPLE, we used oligonucleotide s U133A and U133B chips containing 44792 transcripts. After hybridization of mRNA to the oliginucleotide s raw data was collected and the hybridization intensity for each gene expression is computed as the average of the probes within each probe set of a gene in the chips. Six hundred twenty-four (624) genes were 'extracted' from the metastatic vs. benign tissues with significant p-value < 0.01 for differential expression (*see* TABLE 2 herein below).

The genes from the combined set of probes (U133A and U133B) are ordered by the ABS (t-statistic). For further validation, genes with t-statistics cutoff of 2 or above 2 were selected.

624 genes are disclosed that are significantly altered in cancer tissues. In particular cases, the results are consistent with previous findings of the upregulation and down regulation of particular genes in prostate cancer (Dhanasekaran et al., *Nature* 412:822-826, 2001; Luo et al., *Cancer Res.* 61:4683-4688, 2001; Magee et al., *Cancer Res.* 61:5692-5696, 2001; Welsh et al., *Cancer Res.* 61:5974-5978, 2001; Rubin et al., *J. Amer. Med. Assn.* 287:1662-1670, 2002; Ernst et al., *Am. J. Pathol.* 160:2169-2180, 2002; Sakai et al., *Prostate* 38:278-284, 1999).

According to the present invention, the alteration in the expression profiles of the genes is highly associated with prostate cancer progression and potentially can be useful biomarkers for predicting progression of the cancer.

The validated genes include seven (7) down-regulated genes, and four (4) up-regulated genes. Specifically, the validated down-regulated genes include: Supervillin (SVIL); Proline rich membrane anchor 1 (PRIMA1); TU3A; FLJ14084; KIAA1210; Sorbin and SH3 domain containing 1 (SORBS1); and C21orf63. The validated up-regulated genes include: MARCKS-like protein (MLP); SRY (sex determining region Y)-box 4 (SOX4); Fatty acid binding protein 5 (FABP5); and MAL2.

Validation confirmed the -based strong inverse correlation in the expression of all seven down-regulated genes (SVIL, PRIMA1, TU3A, FLJ14084; KIAA1210, SORBS1 and C21orf63) with progression of prostate cancer.

Likewise, validation confirmed the microarray-based correlation of increased expression, in Gleason grade 6 and Gleason grade 9 tissues, for all four upregulated genes (MLP, SOX4, FABP5 and MAL2).

Furthermore, the mRNA expression levels of the FLJ14084, SVIL, KIAA1210, PRIMA1 and TU3A genes in prostate cancer cell lines were restored by treatment of cells with 5-aza-2'-deoxycytidine, an inhibitor of DNA methylation, thereby implicating the transcriptional silencing of these genes by methylation in prostate cancer cells, and indicating that genomic DNA methylation is correlated with prostate tumorigenesis.

According to aspects of the present invention, the altered methylation and/or expression of these genes provide for novel diagnostic and/or prognostic assays for detection of precancerous and cancerous lesions of the prostate. The inventive compositions and methods have great utility as independent and/or supplementary approaches to standard histopathological work-up of precancerous and cancerous lesions of the prostate.

SVIL, a 205-kDa actin-binding protein is characterized as coregulator of the androgen receptor. Supervillian has shown to enhance the androgen receptor transactivation in muscle and other cells.

PRIMA1 is a membrane anchor of acetylcholinesterase. As a tetramer, acetylcholinesterase is anchored to the basal lamina of the neuromuscular junction and to the membrane of neuronal synapses. PRIMA anchors acetylcholinesterase in brain and muscle cell membranes.

TU3A gene is located in a commonly deleted region on 3p14.3-p14.2 in renal cell carcinoma. This gene encodes a protein consisting of 144 amino acids.

FLJ14084 and KIAA1210 genes maps on chromosome X at positions Xq22.1 and Xq24. The functions of these genes are unknown.

SORBS1 is an actin binding cytoskeletal protein involved in cell-matrix adhesion.

C21orf63 (human chromosome 21 open reading frame 63) encodes a protein with two D-galactoside/L-rhamnose binding SUEL domains.

MLP a macrophage myristolylated alanine rich C kinase substrate related protein encodes a MARCKS-like protein, a substrate for PKC.

SOX4 is a HMG (high mobility group) box 4 transcription factor involved in the regulation of embryonic development and in the determination of cell fate.

FABP5 (psoriasis associated) belongs to a family of small, highly conserved, cytoplasmic proteins that bind long-chain fatty acids and other hydrophobic ligands. FABPs roles include fatty acid uptake, transport and metabolism.\

MAL2, an integral membrane protein of the MAL family, is an essential component of the machinery necessary for the indirect transcytotic route of apical transport in hepatoma HepG2 cells. The gene MAL2 is localized to chromosomal band 8q23 and potentially implicates TPD52-like proteins in vesicle transport.

Specifically, eleven (11) genes were validated by real time PCR to confirm the . The Kruskal-Wallis global test was done with the real-time quantitative analysis for all the genes (FIGURES 4-14).

FIGURES 4-14 show, respectively, the expression levels of eleven genes (PRIMA1, TU3A, KIAA1210, FLJ14084; SVIL, SORBS1, C21orf63, MAL2, FABP5, SOX4 and MLP) as validated by Taqman real-time PCR analysis (including the Kruskal-Wallis global test) in 40 prostate tissue samples and expressed as the relative fold increase (MAL2, FABP5, SOX4 and MLP; FIGURES 11-14, respectively) or decrease (PRIMA1, TU3A, KIAA1210, FLJ14084; SVIL, SORBS1 and C21orf63; FIGURES 4-10, respectively) in the mRNA expression over the adjacent benign tissues after normalization to the house-keeping gene GAPDH mRNA levels. Mean and standard deviations are shown on the right. This real-time PCR data validates results from the instant -based expression analysis.

Therefore, as shown in FIGURES 4-10 and Table 3, a significant decrease in the expression of the PRIMA1, TU3A, KIAA1210, FLJ14084; SVIL, SORBS1 and C21orf63 genes was confirmed in metastatic *versus* organ confined and localized tumors compared to benign tissues ($p<0.0004$), and the decrease in the expression in prostate tumors indicates that they may play an important role in the development and progression of prostate cancer.

Validation of the MAL2, FABP5, SOX4 and MLP genes revealed a significant upregulation in the expression in Gleason grade 6 and Gleason grade 9 tissues compared to the metastatic tissues (FIGURES 11-14 and Table 3). The increase in mRNA levels of MAL2, MLP, SOX4 and FABP5 in cancer tissues indicates a role in prostate cancer development.

Transcriptional silencing. Additionally, to study the possibility of transcriptional silencing of the above-described down-regulated genes in prostate cancer, prostate cancer cells (LAPC4, LNCaP and PC3 cell lines) were treated with an inhibitor of DNA methylation, 5-aza-2-deoxycytidine (5-Aza-CdR) (*see* Vanaja et al 2003, *supra*, for methodology) (see FIGURES 15-19, for analysis the FLJ14084, SVIL, KIAA1210, PRIMA1 and TU3A genes, respectively)

FIGURE 15 shows that a significant increase in the expression of FLJ14084 mRNA levels was found in all three prostate cancer cells tested.

FIGURES 16 and 18, respectively, show that Supervillin (SVIL) and PRIMA1 exhibited a significant increase in LAPC4 and PC3 cells but not in LACaP.

FIGURES 17 and 19, respectively, show that KIAA1210 mRNA levels were increased in LAPC4 and LNCaP cells, and that TU3A expression levels were significantly increased in LNCaP cells but not in LAPC4 and PC3 cells.

The increase in the mRNA levels of FLJ14084, SVIL, PRIMA1, KIAA1210 and TU3A by 5-Aza-CdR indicates that the gene is silenced by methylation in prostate cancer cells.

Therefore, mRNA expression profiling with oligonucleotide s identified 624 genes, the differential expression of which distinguishes and characterizes prostate cancer and benign prostatic tissues.

A decrease in the expression of seven downregulated genes was confirmed by real-time PCR analysis and validates a statistically significant correlation with prostate cancer progression. Restoration of the mRNA expression of FLJ14084, SVIL, KIAA1210, PRIMA1 and TU3A by a DNA methylation inhibitor indicates that the genes are, at least in part, silenced by DNA methylation.

Increase of SOX4, MLP, FABP5 and MAL2 levels indicates a role in development and/or progression of prostate cancer.

Significantly, this is the first study to identify alteration in the expression of these eleven genes in patients with advanced prostate cancer, and they may serve as an independent and/or adjunct marker of prostate cancer aggressiveness.

TABLE 1. Prostate tissue samples with preoperative PSA values at diagnosis, Gleason histological scores, and metastasis status of the tissues. A total of 40 prostate tissues were used to study the gene expression profiling.

Grade	ID	Age	% of tumor	Preop PSA	TNM (97)	Ploidy	METS
Grade 6	,1	55	90	9.4	T2b,N0-	Diploid	
	2	50	80	7.5	T2b,N0-	Tetraploid	
	3	57	80	10.3	T2b,N0-	Diploid	
	4	67	80	16.7	T2b,N0-	Diploid	
	5	68	90	8.1	T2a,N0-	Diploid	
	6	71	95	17.1	T2b,N1+	Aneuploid	
	7	61	80	5.2	T2b,N0+	Diploid	
	8	71	100	41	T2b,N0+	Diploid	
	9	65	75	7	T2a,N0+	Diploid	
	10	51	70	14.3	T2b,N0+	Diploid	
	11	66	90	23.5	T2b,N0+	Tetraploid	
	12	65	80	6.5	T2b, NO-	Diploid	
Grade 9	1	67	90	21.6	T3aN0	Tetraploid	
	2	65	80	29.4	T3bN0	Tetraploid	
	3	65	75	24.9	T3bN0	Tetraploid	
	4	54	80	50	T3bN0	Tetraploid	
	5	59	75	25.8	T3bN0	Diploid	
	6	61	90	3.5	T3aN0	Aneuploid	
	7	72	90	2.5	T3bN0	Tetraploid	
	8	57	90	0.22	T3aN0	Aneuploid	

Grade	ID	Age	% of tumor	Preop PSA	TNM (97)	Ploidy	METS
	9	71	70	8.9	T3aN0	Diploid	
	10	66	100	4.5	T3a,N0+	Diploid	
	11	65	75	6.69	T3b,N0+	Tetraploid	
	12	76	100	7.6	T3b,N1+	Diploid	
	13	71	100	467	T3b,N0+	Aneuploid	
	14	69	70	5.6	T3b,No+	Diploid	liver,bone
	15	66	100	2.9	T3b,N1-	Aneuploid	
Metastatic	M 1	62	90		Metastatic lesion to liver		
	M 2				Peritoneal implant		
	M 3				Lymph node		
	M 4				Lymph node		
	M 5	68	90	8.9	Metastatic prostate cancer in lung.		

TABLE 2. Differential expression (relative to benign tissue) of 624 significantly regulated genes in 40 prostate tissue samples. The expression is computed as the average of the probes within each probe set of a gene in the chips. The 624 genes were 'extracted' from the metastatic vs. benign tissues with significant p-value < 0.01. The genes from the combined set of probes (U133A and U133B) were ranked by the ABS (t-statistic). Genes were selected for further study based on a t-statistics cutoff of 2 or above 2. A negative t-statistic value indicates a decrease in, and positive indicates an increase in the expression of genes in cancer tissues. The fold-change in the expression of genes in Metastatic, Gleason grade 9 and Gleason grade 6 as compared to adjacent benign tissues are shown at the right.

Affymetrix ProbeSetName	Genbank	Unigene	Metastatic p-value	Metastatic t-statistic	Gene	Fold Change		
						Met-Nrml	G9 - Nrml	G6 - Nrml
202274_at	NM_001615.2	Hs.378774	0	-22.5051	ACTG2	0.053803311	0.275524014	0.321307046
201496_x_at	AI889739	Hs.78344	0	-16.3756	MYH11	0.092513093	0.311334938	0.392683897

Affymetrix ProbeSetName	Genbank	Unigene	Metastatic p-value	Metastatic t-statistic	Gene	Fold Change Met-Nml	G9 - Nml	G6 - Nml
200621_at	NM_004078.1	Hs.108080	0	-15.4063	CSRP1	0.196300809	0.391723864	0.405003189
214027_x_at	AA889653	Hs.279604	0	-15.1949	DES	0.220582131	0.453197127	0.437336656
202555_s_at	NM_005965.1	Hs.211582	0	-14.5834	MYLK	0.106681549	0.320630291	0.341562201
205564_at	NM_007003.1	Hs.95420	0	-14.42	GAGEC1	0.261255045	0.508938854	0.677749388
203951_at	NM_001299.1	Hs.21223	0	-14.2117	CNN1	0.112656911	0.363696874	0.354889317
212730_at	AK026420.1	Hs.10587	0	-13.1138	DMN	0.140553471	0.332814198	0.356094906
207876_s_at	NM_001458.1	Hs.58414	0	-12.8903	FLNC	0.474950906	0.597498448	0.621066165
204083_s_at	NM_003289.1	Hs.300772	0	-12.1739	TPM2	0.149184376	0.39284232	0.405764156
201058_s_at	NM_006097.1	Hs.9615	0	-12.1029	MYL9	0.11968876	0.321698372	0.332586079
205547_s_at	NM_003186.2	Hs.433399	0	-12.0177	TAGLN	0.106828219	0.406442173	0.349395924
200974_at	NM_001613.1	Hs.195851	0	-11.5691	ACTA2	0.17792117	0.463927526	0.40713061
209948_at	U61536.1	Hs.93841	0	-11.5427	KCNMB1	0.362212251	0.556744547	0.560864417
201820_at	NM_000424.1	Hs.433845	0	-11.3437	KRT5	0.280032698	0.384279156	0.429128229
226303_at	AA706788	Hs.46331	0	-10.9808	PGM5	0.234867491	0.444812189	0.531081579
203766_s_at	NM_012134.1	Hs.79386	0	-10.5978	LMOD1	0.258393922	0.503828085	0.466892497
205549_at	NM_006198.1	Hs.80296	0	-10.3913	PCP4	0.135604995	0.384014747	0.345619693
226523_at	AI082237	Hs.32978	0	-10.3433	PCSK7	0.540871217	0.722179949	0.625803398
211737_x_at	BC005916.1	Hs.44	0	-10.1922	PTN	0.372578608	0.706509794	0.925406566
221667_s_at	AF133207.1	Hs.111676	0	-10.0549	H11	0.28591921	0.432577624	0.498592093
202504_at	NM_012101.1	Hs.82237	0	-9.8229	TRIM29	0.362228754	0.451921947	0.466335609
211276_at	AF063606.1	Hs.356068	0	-9.7461	MY048	0.518494652	0.718165729	0.697505604
205856_at	NM_015865.1	Hs.171731	0	-9.4026	SLC14A1	0.423229445	0.555799182	0.581379854
213371_at	AI803302	Hs.49998	0	-9.1891	LDB3	0.577603464	0.705513913	0.745367895
219478_at	NM_021197.1	Hs.36688	0	-8.9672	WFDC1	0.306657563	0.57816262	0.539783258
202566_s_at	AF051851.1	Hs.154567	0	-8.9067	SVIL	0.56810571	0.664300873	0.616844465
225721_at	AI658662	Hs.24192	0	-8.7832	SYNPO2	0.211455588	0.477462293	0.438029507
37005_at	D28124	Hs.76307	0	-8.7348	NBL1	0.319533792	0.515936194	0.641274562
204400_at	NM_005864.1	Hs.24587	0	-8.7168	EFS	0.570344842	0.691853688	0.795672591
203370_s_at	NM_005451.2	Hs.102948	0	-8.606	ENIGMA	0.482541378	0.692765088	0.579424908
210297_s_at	U22178.1	Hs.433392	0	-8.564	MSMB	0.049869989	0.166938871	0.444403085
230595_at	BF677651	---	0	-8.5487	FLJ40899	0.387347112	0.507947468	0.570499488
210987_x_at	M19267.1	Hs.77899	0	-8.4458	TPM1	0.287632225	0.446692011	0.445839571
213992_at	AI889941	Hs.408	0	-8.3452	COL4A6	0.603412488	0.723897608	0.730134432
241350_at	AL533913	Hs.86999	0	-8.3425	LOC283807	0.666081008	0.763231436	0.747271248
221246_x_at	NM_018274.2	Hs.351432	0	-8.3418	TNS	0.526103794	0.675841286	0.622485396

Affymetrix ProbeSetName	Genbank	Unigene	Metastatic p-value	Metastatic t-statistic	Gene	Fold Change		
						Met-Nml	G9 - Nml	G6 - Nml
204734_at	NM_002275.1	Hs.80342	0	-8.3269	KRT15	0.236632551	0.357945338	0.416315147
223623_at	AF325503.1	Hs.43125	0	-8.2904	ECRG4	0.396258177	0.707056669	0.606054804
241879_at	AW511222	Hs.296326	0	-8.2151	sp:P39189	0.582477482	1.020217149	0.915877876
205316_at	BF223679	Hs.118747	0	-8.1393	SLC15A2	0.511602561	0.88612165	1.096600868
205132_at	NM_005159.2	Hs.118127	0	-8.1281	ACTC	0.445183351	0.562177326	0.635825598
218087_s_at	NM_015385.1	Hs.108924	0	-8.0964	SORBS1	0.196441183	0.476915472	0.483022062
203296_s_at	NM_000702.1	Hs.34114	0	-8.0632	ATP1A2	0.546867898	0.673105514	0.71171158
219090_at	NM_020689.2	Hs.12321	0	-7.877	SLC24A3	0.630015865	0.827470089	0.756875262
209167_at	AF016004.1	Hs.5422	0	-7.8638	GPM6B	0.506791341	0.708935715	0.729964766
202822_at	AL044018	Hs.180398	0	-7.7949	LPP	0.414861492	0.665931121	0.621661858
227826_s_at	AW138143	Hs.156880	0	-7.7459	IMAGE:4791597	0.202170331	0.483537908	0.449814255
209863_s_at	AF091627.1	Hs.137569	0	-7.7045	TP73L	0.480129801	0.577410686	0.582774883
214752_x_at	AI625550	Hs.195464	0	-7.6432	FLNA	0.255719948	0.450881595	0.37282063
201957_at	AF324888.1	Hs.130760	0	-7.4586	PPP1R12B	0.350435619	0.590001393	0.477521857
209270_at	L25541.1	Hs.75517	0	-7.4324	LAMB3	0.658071625	0.709333463	0.717732863
235468_at	AA531287	Hs.11924	0	-7.4106	LOC339162	0.659275233	0.731812864	0.789170886
207390_s_at	NM_006932.1	Hs.149098	0	-7.4075	SMTN	0.283040393	0.441159739	0.389854498
207016_s_at	AB015228.1	Hs.95197	0	-7.3893	ALDH1A2	0.450127957	0.616891031	0.631455824
228232_s_at	NM_014312.1	Hs.112377	0	-7.3768	CTXL	0.617402852	0.751970331	0.822702013
201431_s_at	NM_001387.1	Hs.74566	0	-7.376	DPYSL3	0.44502532	0.658801891	0.583119459
214175_x_at	BE043700	Hs.424312	0	-7.3391	RIL	0.653610738	0.744219621	0.758834964
204491_at	R40917	Hs.172081	0	-7.3239	PDE4D	0.657929279	0.771456315	0.760289946
205265_s_at	NM_005876.1	Hs.21639	0	-7.3185	APEG1	0.650580959	0.826154763	0.735291274
227827_at	AW138143	Hs.156880	0	-7.2467	IMAGE:4791597	0.205405593	0.486158058	0.444403587
219167_at	NM_016563.1	Hs.27018	0	-7.218	RIS	0.551508072	0.70270956	0.677791849
221584_s_at	U11058.2	Hs.89463	0	-7.1988	KCNMA1	0.465638173	0.713011709	0.740351333
204990_s_at	NM_000213.1	Hs.85266	0	-7.1772	ITGB4	0.640435624	0.673685098	0.651352082
200906_s_at	AK025843.1	Hs.194431	0	-7.0866	KIAA0992	0.559112821	0.708081908	0.639547875
227727_at	H15920	Hs.118513	0	-7.0704	MGC21621	0.503312422	0.723243606	0.684342661
213675_at	W61005	Hs.424272	0	-6.9873	FLJ46049 fis	0.648174796	0.82023855	0.773977519
216264_s_at	X79683.1	Hs.90291	0	-6.9284	LAMB2	0.612076466	0.754958113	0.76493073
204931_at	NM_003206.1	Hs.78061	0	-6.8922	TCF21	0.505430709	0.809029779	0.826637353
203585_at	NM_007150.1	Hs.16622	0	-6.8917	ZNF185	0.505830837	0.615699181	0.615001687
214505_s_at	AF220153.1	Hs.239069	0	-6.8661	FHL1	0.354969836	0.565246533	0.478041452
225524_at	AU152178	Hs.5897	0	-6.8558	ANTXR2	0.409339229	0.677654832	0.830447277

Affymetrix ProbeSetName	Genbank	Unigene	Metastatic p-value	Metastatic t-statistic	Gene	Fold Change Met-Nrml	G9 - Nrml	G6 - Nrml
208789_at	BC004296.1	Hs.29759	0	-6.7973	PTRF	0.48382159	0.606341207	0.598833579
229578_at	AA716165	Hs.134933	0	-6.7872	JPH2	0.611911671	0.753071229	0.719712403
204069_at	NM_002398.1	Hs.170177	0	-6.7853	MEIS1	0.477877704	0.742008585	0.615699332
204268_at	NM_005978.2	Hs.38991	0	-6.6896	S100A2	0.644792961	0.724799993	0.709511387
203687_at	NM_002996.1	Hs.80420	0	-6.6537	CX3CL1	0.604335928	0.70778563	0.696839146
226047_at	N66571	Hs.432673	0	-6.6187	MRVI1	0.54659298	0.764619642	0.704681576
229339_at	AI093327	Hs.42128	0	-6.6142	MYOCD	0.652300902	0.762761259	0.742382465
204455_at	NM_001723.1	Hs.198689	0	-6.6119	BPAG1	0.437282846	0.553091326	0.529050223
227188_at	AI744591	Hs.30156	0	-6.5874	C21ORF63	0.627711098	0.742259445	0.734336678
212236_x_at	Z19574	Hs.2785	0	-6.5682	KRT17	0.244018067	0.354016876	0.391642401
211864_s_at	AF207990.1	Hs.234680	0	-6.5289	FER1L3	0.638621974	0.717399972	0.721878751
221541_at	AL136861.1	Hs.262958	0	-6.4859	DKFZP434B044	0.41721507	0.599924344	0.641831035
227688_at	AK022128.1	Hs.65366	0	-6.4684	KIAA1495	0.633294812	0.814358954	0.815206337
219685_at	NM_021637.1	Hs.45140	0	-6.4435	FLJ14084	0.586063163	0.717268449	0.72677563
212148_at	BF967998	Hs.21851	0	-6.4376	PBX1	0.42188315	0.739252199	0.739111604
203892_at	NM_006103.1	Hs.2719	0	-6.4309	WFDC2	0.442888969	0.528585158	0.527606737
206938_at	NM_000348.1	Hs.1989	0.0001	-6.2511	SRD5A2	0.645321331	0.709715832	0.700927697
203453_at	NM_001038.1	Hs.2794	0.0001	-6.2336	SCNN1A	0.398698168	0.714327568	0.59825747
208131_s_at	NM_000961.1	Hs.302085	0.0001	-6.2334	PTGIS	0.55428096	0.707921871	0.663877631
225328_at	BF693502	Hs.6630	0.0001	-6.2159	FBXO32	0.554087468	0.725502261	0.670659094
229947_at	AI088609	Hs.98558	0.0001	-6.215	FLJ26876 fis	0.339316921	0.587017326	1.271328015
209283_at	AF007162.1	Hs.391270	0.0001	-6.2045	CRYAB	0.48330264	0.605081516	0.606280623
238877_at	BE674583	Hs.102408	0.0001	-6.1438	EYA4	0.657537486	0.800115833	0.76159609
212647_at	NM_006270.1	Hs.9651	0.0001	-6.0582	RRAS	0.654375113	0.704479436	0.746177433
201787_at	NM_001996.1	Hs.79732	0.0001	-5.9802	FBLN1	0.464771633	0.665149327	0.666501329
202054_s_at	NM_000382.1	Hs.159608	0.0001	-5.9675	ALDH3A2	0.596718306	0.72605588	0.839818723
201022_s_at	NM_006870.2	Hs.82306	0.0001	-5.9596	DSTN	0.469263509	0.735850647	0.812634097
204418_x_at	NM_000848.1	Hs.279837	0.0001	-5.9382	GSTM2	0.48069341	0.583085524	0.513812759
203571_s_at	NM_006829.1	Hs.74120	0.0001	-5.9171	APM2	0.341804932	0.546438229	0.568429103
218418_s_at	NM_015493.1	Hs.284208	0.0001	-5.9077	KIAA1518	0.584255705	0.705547521	0.626408504
221004_s_at	NM_030926.1	Hs.111577	0.0001	-5.8947	ITM2C	0.653257154	0.736561823	0.83311969
209651_at	BC001830.1	Hs.25511	0.0001	-5.8551	TGFB1I1	0.458573659	0.578853882	0.600982832
242447_at	AI656180	Hs.359230	0.0001	-5.7774	IMAGE2243078	0.558245981	0.699712197	0.721118844
225990_at	BF343163	Hs.339352	0.0001	-5.7608	BOC	0.554458141	0.856383743	0.767316078
200824_at	NM_000852.2	Hs.226795	0.0001	-5.7489	GSTP1	0.62528976	0.713573655	0.619455086

Affymetrix ProbeSetName	Genbank	Unigene	Metastatic p-value	Metastatic t-statistic	Gene	Fold Change		
						Met-Nrml	G9 - Nrml	G6 - Nrml
220765_s_at	NM_017980.1	Hs.127273	0.0001	-5.7238	LIMS2	0.583795105	0.720887886	0.650878707
218980_at	NM_025135.1	Hs.288841	0.0001	-5.6835	KIAA1695	0.555775824	0.739032946	0.63430201
226755_at	AI375939	Hs.301885	0.0001	-5.652	NPC-A-5	0.504552312	0.607434268	0.586917627
212992_at	AI935123	Hs.57548	0.0002	-5.6427	C14ORF78	0.564503996	0.748557853	0.700982305
212233_at	AL523076	Hs.82503	0.0002	-5.6365	MAP1B	0.44160083	0.750965592	0.557666109
206104_at	NM_002202.1	Hs.505	0.0002	-5.6175	ISL1	0.575277922	0.881067783	0.809109438
204163_at	NM_007046.1	Hs.63348	0.0002	-5.6011	EMILIN1	0.634511395	0.758346546	0.684017738
227742_at	AI638295	Hs.353146	0.0002	-5.5979	CLIC6	0.670703561	0.790469535	0.748444013
202949_s_at	NM_001450.1	Hs.8302	0.0002	-5.5713	FHL2	0.415411095	0.601046867	0.508834921
225609_at	AI659927	Hs.6634	0.0002	-5.546	DKFZP564O0823	0.395102331	0.525825047	0.676752728
228640_at	BE644809	Hs.339315	0.0002	-5.5441	PCDH7	0.480531518	0.688388165	0.697218477
220595_at	NM_013377.1	Hs.380044	0.0002	-5.5383	DKFZP434B0417	0.57489509	0.73680738	0.725634819
227850_x_at	AW084544	Hs.352987	0.0002	-5.4802	CDC42EP5	0.477969665	0.596031808	0.968440186
226304_at	AA563621	Hs.351558	0.0002	-5.4353	FLJ32389	0.530655476	0.6934539	0.754666976
209291_at	NM_001546.1	Hs.34853	0.0002	-5.4154	ID4	0.455232047	0.721342896	0.566598287
215333_x_at	X08020.1	Hs.301961	0.0002	-5.3931	GSTM1	0.592136213	0.684406135	0.62699488
216331_at	AK022548.1	Hs.74369	0.0002	-5.3927	ITGA7	0.619618876	0.766675236	0.668484029
226103_at	AF114264.1	Hs.216381	0.0002	-5.3885	NEXILIN	0.525120912	0.768419067	0.703204986
235342_at	AI808090	Hs.159425	0.0002	-5.3861	SPOCK3	0.484383621	0.779581929	0.754636038
207480_s_at	NM_020149.1	Hs.104105	0.0002	-5.3838	MEIS2	0.400172683	0.620471855	0.648818113
214724_at	AF070621.1	Hs.61408	0.0002	-5.3704	SECP43	0.581948345	0.79632702	0.894707932
204894_s_at	NM_003734.2	Hs.198241	0.0002	-5.3659	AOC3	0.531891736	0.640777537	0.671825828
204570_at	NM_001864.1	Hs.114346	0.0002	-5.3611	COX7A1	0.583822659	0.688692839	0.667070979
227386_s_at	N63821	Hs.268024	0.0002	-5.3428	DKFZp434C184	0.627647025	0.8254192	0.735537074
203476_at	NM_006670.1	Hs.82128	0.0002	-5.3172	TPBG	0.539920131	0.832778932	0.744024144
204442_x_at	NM_003573.1	Hs.85087	0.0002	-5.3088	LTBP4	0.600486893	0.851972293	0.793883461
225662_at	BE620734	Hs.115175	0.0003	-5.2651	ZAK	0.5234581	0.787517538	0.727394698
212135_s_at	AW517686	Hs.343522	0.0003	-5.2353	ATP2B4	0.636641448	0.732189085	0.630131357
203256_at	NM_001793.1	Hs.2877	0.0003	-5.1976	CDH3	0.647266558	0.766651139	0.779882388
212599_at	AK025298.1	Hs.32168	0.0003	-5.1555	AUTS2	0.590495727	0.899171353	0.757428451
214880_x_at	D90453.1	Hs.325474	0.0003	-5.1539	CALD1	0.652622749	0.773522151	0.728499496
223315_at	AF278532.1	Hs.102541	0.0003	-5.1344	NTN4	0.609203042	0.694091861	0.676407558
237206_at	AI452798	Hs.42128	0.0003	-5.1273	MYCD	0.570277407	0.714769249	0.725829487
200930_s_at	AA156675	Hs.75350	0.0003	-5.1226	VCL	0.57672027	0.704478779	0.716474363
205935_at	NM_001451.1	Hs.155591	0.0003	-5.1024	FOXF1	0.518061956	0.716512988	0.668534803

Affymetrix ProbeSetName	Genbank	Unigene	Metastatic p-value	Metastatic t-statistic	Gene	Fold Change Met-Nml	G9 - Nml	G6 - Nml
227006_at	AA156998	Hs.348037	0.0004	-5.0743	PPP1R14A	0.606215229	0.685190003	0.640681808
231096_at	AA226269	Hs.104215	0.0004	-5.0724	GDEP	0.466191103	0.819874985	1.698312318
228504_at	AI828648	Hs.16757	0.0004	-5.0489	SCN7A	0.660946973	0.894320027	0.869601383
211458_s_at	AF180519.1	Hs.334497	0.0004	-5.0473	GABARAPL3	0.557236207	0.720987448	0.839166916
33767_at	X15306	---	0.0004	-5.0434	NEFH	0.163714626	0.167695942	0.558788587
220617_s_at	NM_018181.1	Hs.380730	0.0004	-5.0414	FLJ10697	0.464292261	0.673385803	0.715109709
225016_at	N48299	Hs.374481	0.0004	-5.0299	APCDD1	0.507423231	0.73987269	0.764999022
209129_at	AF000974.1	Hs.380230	0.0004	-5.014	TRIP6	0.642578679	0.734972834	0.69592588
227088_at	BF221547	Hs.16578	0.0004	-4.9968	FLJ42757	0.440236546	0.753875498	0.690231264
214247_s_at	AU148057	Hs.278503	0.0004	-4.9761	DKK3	0.448464785	0.637052822	0.617597889
219669_at	NM_020406.1	Hs.232165	0.0004	-4.9418	PRV1	0.435784309	0.473668236	0.547428403
209074_s_at	AL050264.1	Hs.8022	0.0005	-4.9284	TU3A	0.474253246	0.571454355	0.643798262
204686_at	NM_005544.1	Hs.96063	0.0005	-4.9119	IRS1	0.599920666	0.780445838	0.717289768
227194_at	BF106962	Hs.20415	0.0005	-4.8943	FAM3B	0.502784686	1.303068671	2.771161255
203373_at	NM_003877.1	Hs.405946	0.0005	-4.8781	SOCS2	0.503022765	0.836972031	1.070200787
204940_at	NM_002667.1	Hs.85060	0.0005	-4.8415	PLN	0.631681514	0.815827405	0.771310785
206953_s_at	NM_012302.1	Hs.24212	0.0005	-4.8194	LPHN2	0.654350027	0.827603525	0.776672002
204393_s_at	NM_001099.2	Hs.1852	0.0006	-4.8016	ACPP	0.115290032	0.329784847	0.855266897
205609_at	NM_001146.1	Hs.2463	0.0006	-4.7892	ANGPT1	0.657951095	0.764380343	0.776848693
225782_at	BG171064	Hs.339024	0.0006	-4.7743	LOC253827	0.458190603	0.67025752	0.614380899
213568_at	AI811298	Hs.348363	0.0006	-4.7513	OSR2	0.595887145	0.817690588	0.802144853
201462_at	NM_014766.1	Hs.75137	0.0006	-4.7481	KIAA0193	0.620924878	0.797802174	0.734057849
222043_at	AI982754	Hs.75106	0.0006	-4.7308	CLU	0.593038992	0.681315769	0.679106494
230087_at	AI823645	Hs.356130	0.0006	-4.7300	PRIMA1	0.744276908	0.774136798	0.814308813
209763_at	AL049176	Hs.82223	0.0007	-4.6823	NRLN1	0.356878935	0.525822669	0.528249548
225243_s_at	AB046821.1	Hs.4007	0.0007	-4.6812	SLMAP	0.554213615	0.739011846	0.700171981
224811_at	BF112093	Hs.5724	0.0007	-4.6687	IMAGE:5286019	0.466515157	0.725388678	0.638970142
212510_at	AA135522	Hs.82432	0.0007	-4.6621	KIAA0089	0.605080242	0.73255191	0.802961174
218694_at	NM_016608.1	Hs.9728	0.0007	-4.6374	ALEX1	0.602846403	0.707313012	0.772724682
203851_at	NM_002178.1	Hs.274313	0.0007	-4.6139	IGFBP6	0.430883315	0.74596986	0.698725182
208848_at	M30471.1	Hs.78989	0.0008	-4.6038	ADH5	0.663568149	0.777969527	0.908558621
203945_at	NM_001172.2	Hs.172851	0.0008	-4.5889	ARG2	0.655767602	0.814139416	1.070857995
218717_s_at	NM_018192.1	Hs.42824	0.0008	-4.582	MLAT4	0.491323587	0.719755368	1.063083603
203789_s_at	NM_006379.1	Hs.171921	0.0008	-4.5809	SEMA3C	0.41407478	0.713966234	0.812832558
212509_s_at	BF968134	Hs.356623	0.0008	-4.5787	FLJ46603	0.389142337	0.624615411	0.532162455

Affymetrix ProbeSetName	Genbank	Unigene	Metastatic p-value	Metastatic t-statistic	Gene	Fold Change		
						Met-Nrml	G9 - Nrml	G6 - Nrml
205383_s_at	NM_015642.1	Hs.159456	0.0008	-4.5747	ZNF288	0.548989134	0.694480542	0.641379066
207836_s_at	NM_006867.1	Hs.80248	0.0009	-4.5315	RBPMS	0.615089794	0.728032204	0.641435394
212361_s_at	AK000300.1	Hs.374535	0.0009	-4.5291	ATP2A2	0.560457216	0.695746344	0.672952848
201841_s_at	NM_001540.2	Hs.76067	0.0009	-4.5208	HSPB1	0.417356832	0.688393006	0.652979705
231098_at	BF939996	Hs.10263	0.0009	-4.5188	IMAGE:3439264	0.634015979	0.834876525	0.877772325
208637_x_at	BC003576.1	Hs.119000	0.0009	-4.5141	ACTN1	0.507507171	0.670744352	0.696527754
203780_at	AF275945.1	Hs.116651	0.0009	-4.488	EVA1	0.584182656	0.691457443	0.722126066
224710_at	AF322067.1	Hs.301853	0.001	-4.4671	RAB34	0.603159118	0.718491133	0.652709312
205827_at	NM_000729.2	Hs.80247	0.001	-4.462	CCK	0.553054062	0.583055181	0.642464516
209747_at	J03241.1	Hs.2025	0.001	-4.449	TGFB3	0.651515999	0.724745281	0.705691493
202948_at	NM_000877.1	Hs.82112	0.001	-4.4472	IL1R1	0.604437089	0.82106783	1.181763499
227719_at	AA934610	Hs.103262	0.001	-4.4124	MADH9	0.578200978	0.986277084	0.947599385
205413_at	NM_001584.1	Hs.46638	0.001	-4.4076	C11ORF8	0.575640879	0.704424248	0.969192324
205158_at	NM_002937.1	Hs.283749	0.0011	-4.3995	RNASE4	0.553261747	0.725854518	0.920722712
218094_s_at	NM_018478.1	Hs.256086	0.0011	-4.3978	C20ORF35	0.634327286	0.733681563	0.668763089
227183_at	AI417267	Hs.84630	0.0011	-4.3909	FLJ36638	0.476507931	0.748959021	0.510943793
200795_at	NM_004684.1	Hs.75445	0.0012	-4.3223	SPARCL1	0.332891488	0.572497655	0.580836191
201289_at	NM_001554.1	Hs.8867	0.0013	-4.2923	CYR61	0.357935903	0.675898539	0.504255247
209309_at	D80427.1	Hs.71	0.0013	-4.2714	AZGP1	0.188668426	0.411500713	1.225895651
233496_s_at	AV726166	Hs.180141	0.0013	-4.2675	CFL2	0.668714724	0.774968364	0.753424733
219295_s_at	NM_013363.1	Hs.8944	0.0013	-4.2607	PCOLCE2	0.597237277	0.864177696	0.815426915
213110_s_at	AW052179	Hs.169825	0.0013	-4.2602	COL4A5	0.623714985	0.82101802	0.725098366
208937_s_at	D13889.1	Hs.75424	0.0014	-4.2327	ID1	0.340094789	0.424134354	0.368659343
208873_s_at	BC000232.1	Hs.178112	0.0014	-4.2192	DP1	0.648135188	0.856221541	1.050337148
217728_at	NM_014624.2	Hs.275243	0.0014	-4.2167	S100A6	0.485193905	0.623702181	0.541296022
221814_at	BF511315	Hs.17270	0.0015	-4.2012	GPR124	0.621857706	0.752341694	0.704499619
217546_at	R06655	Hs.188518	0.0015	-4.1962	MT1K	0.456798259	0.504132777	0.901930375
232332_at	AI610999	Hs.97594	0.0015	-4.196	KIAA1210	0.563855803	0.627364514	0.635441044
201234_at	NM_004517.1	Hs.6196	0.0015	-4.1911	ILK	0.603354892	0.6840541	0.683440877
232541_at	AK000106.1	Hs.272227	0.0015	-4.1859	FLJ20099	0.552914557	0.849544303	0.615331046
225464_at	N30138	Hs.250705	0.0015	-4.1857	C14ORF31	0.5944659	0.681084121	0.654445794
214898_x_at	AB038783.1	Hs.129782	0.0016	-4.1732	MUC3B	0.667579274	0.73585261	0.758074809
212423_at	AL049949.1	Hs.28264	0.0016	-4.1669	FLJ90798	0.638894251	0.777384156	0.769528281
218552_at	NM_018281.1	Hs.34579	0.0016	-4.1514	FLJ10948	0.588253779	0.87834189	0.833885251
209505_at	AI951185	Hs.374991	0.0016	-4.1505	NR2F1	0.549274414	0.855084544	0.763129922

Affymetrix ProbeSetName	Genbank	Unigene	Metastatic p-value	Metastatic t-statistic	Gene	Fold Change		
						Met-Nrml	G9 - Nrml	G6 - Nrml
213338_at	BF062629	Hs.35861	0.0016	-4.1476	RIS1	0.522606426	0.648514993	0.736649186
201389_at	NM_002205.1	Hs.149609	0.0016	-4.1416	ITGA5	0.606773347	0.600410887	0.58991654
209288_s_at	AL136842.1	Hs.260024	0.0016	-4.1414	CDC42EP3	0.477391739	0.66604325	0.682947642
221958_s_at	AA775681	Hs.250746	0.0017	-4.1363	FLJ23091	0.63702265	0.874469966	1.118857498
209351_at	BC002690.1	Hs.355214	0.0018	-4.095	KRT14	0.411699514	0.433050412	0.549270807
208949_s_at	BC001120.1	Hs.621	0.0019	-4.0458	LGALS3	0.428078808	0.526116633	0.636966353
232224_at	AI274095	Hs.356082	0.0019	-4.0433	MASP1	0.648107552	0.770747674	0.817503851
217168_s_at	AF217990.1	Hs.146393	0.002	-4.0353	HERPUD1	0.582877469	0.698372654	1.125172106
213005_s_at	D79994.1	Hs.77546	0.002	-4.0149	KANK	0.585757723	0.687948638	0.739770133
227623_at	H16409	Hs.298258	0.002	-4.0108	FLJ30478	0.599171183	0.685627452	0.729463584
204464_s_at	NM_001957.1	Hs.76252	0.0022	-3.9793	EDNRA	0.513268454	0.714259369	0.624579225
201300_s_at	NM_000311.1	Hs.74621	0.0023	-3.9405	PRNP	0.506550021	0.673224331	0.718988125
226051_at	BF973568	Hs.55940	0.0023	-3.9309	SELM	0.502400452	0.679612919	0.613157831
228325_at	AI363213	Hs.278634	0.0024	-3.9299	KIAA0146	0.536626452	0.659648909	0.672068485
235518_at	AI741439	Hs.144465	0.0024	-3.9297	SLC8A1	0.639765337	0.838297436	0.79588328
212848_s_at	BG036668	Hs.334790	0.0024	-3.9225	FLJ14675	0.582906821	0.78306189	0.629500001
217023_x_at	AF099143	---	0.0025	-3.904	TPSB2	0.630895637	0.769488455	0.921618372
230577_at	AW014022	Hs.170953	0.0026	-3.8775	sp:P00722	0.53651314	0.596534666	0.865585113
201645_at	NM_002160.1	Hs.289114	0.0028	-3.838	TNC	0.604361212	0.673498683	0.665240809
212805_at	AB0023651	Hs.23311	0.003	-3.796	KIAA0367	0.488940651	0.733752548	0.939729963
212993_at	AA114166	Hs.381190	0.003	-3.791	IMAGE:5311129	0.648379666	0.750751439	0.830305196
201121_s_at	NM_006667.2	Hs.90061	0.003	-3.7858	PGRMC1	0.63646248	0.694566848	0.718897767
235759_at	AI095542	Hs.302754	0.0031	-3.7703	EFCBP1	0.671683695	0.766080043	0.773001887
201667_at	NM_000165.2	Hs.74471	0.0031	-3.7625	GJA1	0.38086039	0.477853618	0.510113877
206070_s_at	AF213459.1	Hs.123642	0.0031	-3.761	EPHA3	0.578192384	1.028434338	0.942403658
209498_at	X16354.1	Hs.50964	0.0032	-3.7594	CEACAM1	0.598189696	0.639236175	0.72565747
222325_at	AW974812	Hs.433049	0.0033	-3.7351	EST386917	0.581645323	0.89684438	0.711318846
203973_s_at	NM_005195.1	Hs.76722	0.0033	-3.7327	KIAA0146	0.340744017	0.4823812	0.484630011
206714_at	NM_001141.1	Hs.111256	0.0034	-3.7184	ALOX15B	0.456757922	0.654700344	1.510641843
202729_s_at	NM_000627.1	Hs.241257	0.0034	-3.712	LTBP1	0.577127404	0.865778815	0.736276457
39248_at	N74607	Hs.234642	0.0036	-3.6776	AQP3	0.442587059	0.573536836	0.776848921
204457_s_at	NM_002048.1	Hs.65029	0.0037	-3.6673	GAS1	0.426786728	0.533346658	0.543269274
204971_at	NM_005213.1	Hs.2621	0.0037	-3.662	CSTA	0.637757056	0.642734275	0.649581736
204284_at	N26005	Hs.303090	0.004	-3.6304	PPP1R3C	0.595267584	0.676600575	0.692761509

Affymetrix ProbeSetName			Metastatic	Metastatic		Fold Change		
	Genbank	Unigene	p-value	t-statistic	Gene	Met-Nrml	G9 - Nrml	G6 - Nrml
202688_at	NM_003810.1	Hs.83429	0.0041	-3.6139	TNFSF10	0.45407484	0.594718895	1.062889226
227917_at	AW192692	Hs.169160	0.0041	-3.6032	DKFZp434N2116	0.664188052	0.871669924	0.737876071
201012_at	NM_000700.1	Hs.78225	0.0043	-3.5822	ANXA1	0.464357655	0.611049645	0.481595141
203824_at	NM_004616.1	Hs.84072	0.0043	-3.5777	TM4SF3	0.41872351	0.762172912	1.070782355
209540_at	NM_000618.1	Hs.85112	0.0043	-3.5768	IGF1	0.604834335	0.931257424	0.877063322
226250_at	AA058578	Hs.104627	0.0044	-3.5722	FLJ10158	0.593260939	0.75021829	0.684919925
222294_s_at	AW971415	Hs.432533	0.0046	-3.5408	RAB27A	0.65139431	0.878147649	1.479261234
218224_at	NM_006029.2	Hs.194709	0.0047	-3.5309	PNMA1	0.569284754	0.703621182	0.725886997
241918_at	AI299378	Hs.351615	0.0047	-3.5304	PCANAP5	0.593365377	0.807994275	1.030091863
209191_at	BC002654.1	Hs.274398	0.0049	-3.5095	TUBB-5	0.576197173	0.641975742	0.599348386
228728_at	BF724137	Hs.255416	0.0049	-3.5031	FLJ21986	0.633648453	0.823222679	0.751461991
235666_at	AA903473	Hs.153717	0.005	-3.5018	sp:P39194	0.613016934	0.857437395	0.832762402
235094_at	AI972661	Hs.30627	0.005	-3.5004	TPM4	0.455653643	0.860778088	0.495363995
203717_at	NM_001935.1	Hs.44926	0.0051	-3.4888	DPP4	0.488633773	0.709272821	1.20340692
212185_x_at	NM_005953.1	Hs.118786	0.0051	-3.4834	MT2A	0.458542813	0.40997157	0.701563388
204908_s_at	NM_005178.1	Hs.31210	0.0051	-3.4813	BCL3	0.644252573	0.665017966	0.71101296
202037_s_at	NM_003012.2	Hs.7306	0.0052	-3.4795	SFRP1	0.542482197	0.861819298	0.687121176
203881_s_at	NM_004010.1	Hs.169470	0.0052	-3.4791	DMD	0.578897468	0.680754017	0.674303926
204326_x_at	NM_002450.1	Hs.380778	0.0052	-3.4728	MT1X	0.448212734	0.386428777	0.735631918
202289_s_at	NM_006997.1	Hs.272023	0.0053	-3.4667	TACC2	0.644209586	0.844559734	1.054515739
225381_at	AW162210	Hs.98518	0.0053	-3.4651	DKFZp686J24156	0.60032367	0.830881356	0.697291406
202133_at	AA081084	Hs.24341	0.0053	-3.4604	TAZ	0.596087848	0.789915793	0.767893734
200799_at	NM_005345.3	Hs.75452	0.0055	-3.4455	HSPA1A	0.525257873	1.022603345	1.350473323
225105_at	BF969397	Hs.301711	0.0055	-3.4396	LOC387882	0.607521675	0.734980308	0.617862671
207935_s_at	NM_002274.1	Hs.74070	0.0058	-3.4118	KRT13	0.608310078	0.789853708	0.656618334
227121_at	AL110204.1	Hs.193784	0.006	-3.3932	DKFZp586K1922	0.595822645	0.75964906	0.71784473
204345_at	NM_001856.1	Hs.26208	0.0061	-3.3833	COL16A1	0.609363288	0.888996822	0.619263593
213156_at	AL049423.1	Hs.16193	0.0061	-3.3813	DKFZp586B211	0.614484055	0.79993889	0.90223163
221935_s_at	AK023140.1	Hs.5997	0.0063	-3.369	MGC34132	0.657690674	0.784246268	0.706166103
203706_s_at	NM_003507.1	Hs.173859	0.0063	-3.3617	FZD7	0.556884887	0.743584977	0.691777229
204793_at	NM_014710.1	Hs.113082	0.0064	-3.3542	GASP	0.640999038	0.770150708	0.676227311
203708_at	NM_002600.1	Hs.188	0.0065	-3.3514	PDE4B	0.618721093	0.695543706	0.740177755
212859_x_at	BF217861	---	0.0065	-3.3489	MT1E	0.431199359	0.381553146	0.798187882
204537_s_at	NM_004961.2	Hs.22785	0.0066	-3.3377	GABRE	0.603828317	0.694224314	0.579239977
202888_s_at	NM_001150.1	Hs.1239	0.0067	-3.3349	ANPEP	0.370164997	0.477411102	1.562801826

Affymetrix ProbeSetName			Metastatic	Metastatic		Fold Change		
	Genbank	Unigene	p-value	t-statistic	Gene	Met-Nrml	G9 - Nrml	G6 - Nrml
202391_at	NM_006317.1	Hs.79516	0.0069	-3.3147	BASP1	0.463230986	0.909162083	0.838497202
204748_at	NM_000963.1	Hs.196384	0.0069	-3.3147	PTGS2	0.391552844	0.610499324	0.522728242
223557_s_at	AB017269.1	Hs.22791	0.0072	-3.2939	TMEFF2	0.478486722	2.173964839	5.040357989
222303_at	AV700891	Hs.292477	0.0072	-3.2925	ETS2	0.500190086	0.644047093	0.477238473
211456_x_at	AF333388.1	Hs.367850	0.0073	-3.2809	MT1H	0.573088114	0.512423936	0.790642019
214696_at	AF070569.1	Hs.417157	0.0074	-3.2775	MGC14376	0.500101466	0.644862395	0.54026883
201599_at	NM_000274.1	Hs.75485	0.0074	-3.2775	OAT	0.560449825	0.628852944	0.653941647
218731_s_at	NM_022834.1	Hs.110443	0.0076	-3.2575	FLJ22215	0.647897719	0.731950802	0.805715513
228188_at	AI860150	Hs.5890	0.0078	-3.2486	FLJ23306	0.612483767	0.730400346	0.657667139
212914_at	AV648364	Hs.356416	0.0079	-3.2399	CBX7	0.672491781	0.780716804	0.690054773
200696_s_at	NM_000177.1	Hs.290070	0.008	-3.2335	GSN	0.483261114	0.725938182	0.568269871
206211_at	NM_000450.1	Hs.89546	0.0083	-3.2081	SELE	0.490034502	0.703663072	0.738701475
242736_at	AI377221	Hs.40528	0.0084	-3.2052	IMAGE:2064085	0.602976013	0.807016023	0.621771592
221024_s_at	NM_030777.1	Hs.305971	0.0084	-3.2046	SLC2A10	0.639798214	0.925382652	1.45314006
205229_s_at	AA669336	Hs.21016	0.0085	-3.1955	COCH	0.620495813	0.854818559	0.735661252
211965_at	X79067.1	Hs.85155	0.0086	-3.1932	ZFP36L1	0.644547553	0.774491249	0.800099031
201560_at	NM_013943.1	Hs.25035	0.0086	-3.1884	CLIC4	0.628588945	0.799632703	0.709436844
202018_s_at	NM_002343.1	Hs.105938	0.0087	-3.1816	LTF	0.0970549	0.17189767	0.307421109
201360_at	NM_000099.1	Hs.304682	0.009	-3.1674	CST3	0.598218982	0.683984155	0.80851963
201369_s_at	NM_006887.1	Hs.78909	0.009	-3.1669	ZFP36L2	0.57332007	0.695638926	0.581983214
225442_at	AI799915	Hs.349303	0.0091	-3.16	DDR2	0.650022328	0.851998744	0.703655507
212724_at	BG054844	Hs.6838	0.0094	-3.138	ARHE	0.524405985	0.610187469	0.578512935
202336_s_at	NM_000919.1	Hs.83920	0.0097	-3.1204	PAM	0.560777596	1.000931184	0.831990839
226189_at	BF513121	Hs.367688	0.0099	-3.1117	IMAGE:4794726	0.628864888	0.787069309	0.733048653
221872_at	AI669229	Hs.82547	0.01	-3.1039	RARRES1	0.33062532	0.489452465	0.499917103
212761_at	AI703074	Hs.348412	0.0102	-3.0937	TCF7L2	0.625047654	0.858457558	0.920807486
243296_at	AA873350	Hs.176554	0.0106	-3.0756	PBEF	0.337927134	0.595396083	0.402394619
241897_at	AA491949	Hs.409080	0.0108	-3.0635	CRL2 precusor	0.628387896	0.855940324	0.600396555
212099_at	AI263909	Hs.204354	0.0112	-3.0404	ARHB	0.402558963	0.5374298	0.46564017
225876_at	T84558	Hs.13804	0.0113	-3.0358	DJ462O23.2	0.526611323	0.650766767	0.893799448
201041_s_at	NM_004417.2	Hs.171695	0.0116	-3.0239	DUSP1	0.451274478	0.665471417	0.688731099
226252_at	AA058578	Hs.104627	0.0116	-3.023	FLJ10158	0.659463151	0.790318933	0.809873125
230788_at	BF059748	Hs.421105	0.0116	-3.0217	GCNT2	0.511752041	0.591273522	0.882837241
200953_s_at	NM_001759.1	Hs.75586	0.0118	-3.0149	CCND2	0.581793396	0.760195445	0.718824623

Affymetrix ProbeSetName	Genbank	Unigene	Metastatic p-value	Metastatic t-statistic	Gene	Fold Change		
						Met-Nrml	G9 - Nrml	G6 - Nrml
33323_r_at	X57348	Hs.184510	0.0118	-3.0142	SFN	0.432853115	0.578204169	0.833345335
204745_x_at	NM_005950.1	Hs.433391	0.0121	-3.0012	MT1G	0.456465598	0.425042163	0.791028837
201150_s_at	NM_000362.2	Hs.245188	0.0121	-3.0004	TIMP3	0.615278264	0.677143574	0.708474175
222162_s_at	AK023795.1	Hs.8230	0.0121	-2.9969	ADAMTS1	0.417960532	0.68593523	0.555010188
213275_x_at	BE875786	Hs.297939	0.0122	-2.9946	CTSB	0.639593717	0.761818881	0.730652349
219682_s_at	NM_016569.1	Hs.267182	0.0124	-2.9839	TBX3	0.523809912	0.886022121	0.970469152
238481_at	AW512787	Hs.404077	0.0125	-2.9807	MGP	0.606083743	1.138279606	0.670651525
209656_s_at	AL136550.1	Hs.8769	0.0128	-2.9684	TM4SF10	0.560601819	0.899717295	0.757505615
201464_x_at	BG491844	Hs.78465	0.013	-2.9584	JUN	0.534670849	0.843913283	0.892066246
202350_s_at	NM_002380.2	Hs.19368	0.0132	-2.9515	MATN2	0.595033679	0.834264276	0.795741335
212768_s_at	AL390736	Hs.273321	0.0133	-2.9456	GW112	0.225216833	0.436827315	0.39398727
209156_s_at	AY029208.1	Hs.159263	0.0133	-2.9454	COL6A2	0.486933097	0.608880847	0.450512965
205692_s_at	NM_001775.1	Hs.66062	0.0134	-2.9417	CD38	0.615350798	0.658995924	0.989624421
222722_at	AV700059	Hs.109439	0.0136	-2.9337	OGN	0.545423692	0.806413801	0.715131507
209016_s_at	BC002700.1	Hs.23881	0.014	-2.9156	KRT7	0.642306014	0.74588737	0.690949593
215111_s_at	AK027071.1	Hs.114360	0.0141	-2.9136	TSC22	0.497282694	0.531538599	0.6436215
209621_s_at	AF002280.1	Hs.135281	0.0142	-2.9109	ALP	0.59333833	0.703856749	0.680927442
242868_at	T70087	Hs.307559	0.0143	-2.9076	IMAGE:80996	0.570499373	0.720976852	0.548770053
218718_at	NM_016205.1	Hs.43080	0.0145	-2.8967	PDGFC	0.570589136	0.759913242	0.671837954
200884_at	NM_001823.1	Hs.173724	0.0145	-2.8963	CKB	0.509732177	0.678228409	0.844919959
212089_at	M13452.1	Hs.377973	0.0152	-2.8724	LMNA	0.665116105	0.739568287	0.679437588
202672_s_at	NM_001674.1	Hs.460	0.0152	-2.8699	ATF3	0.254053258	0.577524204	0.42844299
216598_s_at	S69738.1	Hs.303649	0.0153	-2.8667	CCL2	0.441821303	0.464466134	0.409043457
226769_at	AI802391	Hs.32478	0.0154	-2.8649	LOC387758	0.643967758	1.0013538	0.839964674
209189_at	BC004490.1	Hs.2564?	0.0158	-2.8487	FOS	0.329749759	0.628331868	0.493449262
202286_s_at	J04152	Hs.23582	0.0159	-2.8462	TACSTD2	0.31642776	0.625542647	1.021260519
225673_at	BE908995	Hs.380906	0.0161	-2.8386	LOC91663	0.566986589	0.675313081	0.623314519
205862_at	NM_014668.1	Hs.193914	0.0165	-2.8242	GREB1	0.506078166	0.943886011	1.380149032
205225_at	NM_000125.1	Hs.1657	0.0167	-2.819	ESR1	0.51712671	0.924139409	0.697838254
231783_at	AI500293	Hs.247917	0.0174	-2.7963	CHRM1	0.641574237	0.764137428	1.312516824
201694_s_at	NM_001964.1	Hs.326035	0.0174	-2.7957	EGR1	0.39646573	0.679207349	0.566237865
213428_s_at	AA292373	Hs.108885	0.0177	-2.7862	COL6A1	0.56253883	0.690206606	0.489695051
209369_at	M63310.1	Hs.1378	0.0182	-2.7707	ANXA3	0.643888077	0.907333193	1.231309972
224894_at	BF210049	Hs.84520	0.0184	-2.7634	YAP1	0.607783703	0.821687742	0.748843462
208763_s_at	AL110191.1	Hs.75450	0.0185	-2.7619	DSIPI	0.610365851	0.729534861	0.802532704

Affymetrix ProbeSetName	Genbank	Unigene	Metastatic p-value	Metastatic t-statistic	Gene	Fold Change		
						Met-Nrml	G9 - Nrml	G6 - Nrml
244239_at	AI887306	Hs.137221	0.0194	-2.7355	YN63H06	0.618590896	0.795484734	0.676415916
201425_at	NM_000690.1	Hs.195432	0.0199	-2.7205	ALDH2	0.64506947	0.71496059	0.871943306
217165_x_at	M10943	Hs.381097	0.0199	-2.7204	MT1F	0.532277831	0.459410851	0.95574968
201531_at	NM_003407.1	Hs.343586	0.0201	-2.7164	ZFP36	0.368822278	0.573326486	0.51833161
201236_s_at	NM_006763.1	Hs.75462	0.0202	-2.7111	BTG2	0.449196974	0.574666196	0.564492749
225945_at	BF219240	Hs.115659	0.0204	-2.7073	VIK	0.63857255	0.692757333	0.701380412
202489_s_at	BC005238.1	Hs.301350	0.0205	-2.705	FXYD3	0.413544476	0.691155271	1.267793962
204719_at	NM_007168.1	Hs.38095	0.0209	-2.693	ABCA8	0.565139968	0.757214801	0.707955742
217967_s_at	AF288391.1	Hs.48778	0.0209	-2.6929	C1ORF24	0.543959386	0.73063063	1.104433103
215078_at	AL050388.1	Hs.372783	0.0211	-2.687	SOD2	0.647668168	0.732598208	0.703135648
225557_at	AI091372	Hs.6607	0.0212	-2.6843	AXUD1	0.53852929	0.664192806	0.633086763
204259_at	NM_002423.2	Hs.2256	0.0215	-2.6775	MMP7	0.450118957	0.7288099	0.768253699
205960_at	NM_002612.1	Hs.8364	0.0215	-2.6766	PDK4	0.609608362	0.706936283	0.617091029
209210_s_at	Z24725.1	Hs.75260	0.0219	-2.6683	PLEKHC1	0.549014436	0.638717949	0.609727499
209101_at	M92934.1	Hs.75511	0.0223	-2.6578	CTGF	0.451024698	0.732153169	0.510263768
226506_at	AI742570	Hs.380149	0.0223	-2.6567	FLJ13710	0.659953836	0.709491486	0.758949079
209118_s_at	AF141347.1	Hs.433394	0.0232	-2.6349	TUBA3	0.668082045	0.768266303	0.670094444
213791_at	NM_006211.1	Hs.93557	0.0237	-2.6238	PENK	0.649165182	0.735398814	0.732302884
212230_at	AL576654	—	0.024	-2.6149	PPAP2B	0.548857227	0.589286375	0.61198091
217744_s_at	NM_022121.1	Hs.303125	0.0242	-2.6111	PIGPC1	0.636297335	0.789650873	0.957541661
201005_at	NM_001769.1	Hs.1244	0.0245	-2.605	CD9	0.471999699	0.789958319	1.068501023
227399_at	AI754423	Hs.367211	0.0251	-2.5903	LOC51159	0.56959877	0.943253306	1.140816664
237077_at	AI821895	Hs.433060	0.0254	-2.5844	IMAGE:1203949	0.585987134	0.846219403	0.980927952
202340_x_at	NM_002135.1	Hs.1119	0.0264	-2.5621	NR4A1	0.348025216	0.674634071	0.50042662
203140_at	NM_001706.1	Hs.155024	0.0265	-2.5597	BCL6	0.653995843	0.755613259	0.672169483
227642_at	AI928242	Hs.119903	0.0266	-2.5575	TFCP2L1	0.641596799	0.73268621	0.668940723
213931_at	AI819238	Hs.180919	0.0282	-2.5249	pir:A40227	0.629101722	0.781558812	0.616683305
217775_s_at	NM_016026.1	Hs.179817	0.0286	-2.5171	RDH11	0.464165784	0.77978021	1.670415923
213564_x_at	BE042354	Hs.234489	0.0289	-2.5125	LDHB	0.487639647	0.607360774	0.629709594
201650_at	NM_002276.1	Hs.182265	0.03	-2.4907	KRT19	0.556260378	0.552100901	0.58183457
209304_x_at	AF087853.1	Hs.110671	0.0306	-2.4802	GADD45B	0.527433735	0.667118834	0.580847272
243618_s_at	BF678830	Hs.382367	0.0306	-2.4797	LOC152485	0.604180806	0.769951673	0.860931014
240221_at	AV704610	Hs.318381	0.031	-2.4725	CSNK1A1	0.659752573	0.903938631	0.647440833
201105_at	NM_002305.2	Hs.382367	0.0312	-2.4686	LGALS1	0.641063556	0.664405546	0.526293118

Affymetrix ProbeSetName	Genbank	Unigene	Metastatic p-value	Metastatic t-statistic	Gene	Fold Change		
						Met-Nml	G9 - Nml	G6 - Nml
224917_at	BF674052	Hs.374415	0.032	-2.4542	VMP1	0.417797614	0.725339183	0.407411034
222927_s_at	AW295812	Hs.98927	0.032	-2.454	LMAN1L	0.587807901	0.802616467	0.755345307
212665_at	AL556438	Hs.12813	0.0323	-2.4486	DKFZP434J214	0.523667633	0.624272209	0.616181214
224755_at	BE621524	Hs.8203	0.0326	-2.4437	SMBP	0.648166532	0.885971012	0.980484508
201631_s_at	NM_003897.1	Hs.76095	0.035	-2.404	IER3	0.511124962	0.534169945	0.466723395
221841_s_at	BF514079	Hs.376206	0.0355	-2.3961	KLF4	0.444530205	0.685266095	0.582181416
212097_at	AU147399	Hs.74034	0.0372	-2.3686	CAV1	0.672011287	0.525135392	0.575693007
207826_s_at	NM_002167.1	Hs.76884	0.0374	-2.3669	ID3	0.66544141	0.686424697	0.588659692
36711_at	AL021977	Hs.51305	0.0379	-2.3589	MAFF	0.433687817	0.557218356	0.563652161
202720_at	NM_015641.1	Hs.165986	0.0396	-2.3343	TES	0.644177594	0.688210529	0.698168263
202768_at	NM_006732.1	Hs.75678	0.0399	-2.3293	FOSB	0.278626863	0.557553338	0.388079334
223218_s_at	AB037925.1	Hs.301183	0.04	-2.3274	MAIL	0.5298983	0.81241416	0.445748711
203962_s_at	NM_006393.1	Hs.5025	0.0417	-2.304	NEBL	0.668593378	0.788135019	0.747562737
212531_at	NM_005564.1	Hs.204238	0.0428	-2.2902	LCN2	0.246089432	0.278320044	0.355266869
205251_at	NM_022817.1	Hs.153405	0.0444	-2.2687	PER2	0.633196234	0.671066633	0.624644315
209184_s_at	BF700086	Hs.143648	0.0453	-2.2571	IRS2	0.609218577	0.909010722	0.812757521
205319_at	NM_005672.1	Hs.423634	0.0481	-2.2232	PSCA	0.578225484	0.829291736	0.87744188
201312_s_at	NM_003022.1	Hs.14368	0.0515	-2.1839	SH3BGRL	0.552399851	0.754499178	0.836452923
205207_at	NM_000600.1	Hs.93913	0.0523	-2.1756	IL6	0.593094851	0.684302598	0.592307215
206260_at	NM_003241.1	Hs.2387	0.0524	-2.1739	TGM4	0.259043972	0.32178001	0.347372965
211753_s_at	BC005956.1	Hs.105314	0.0525	-2.1733	RLN1	0.553157866	1.243044777	1.980477424
213503_x_at	BE908217	Hs.217493	0.0527	-2.1708	ANXA2	0.635697023	0.542468458	0.54146373
225344_at	AL035689	Hs.339283	0.053	-2.1678	NCOA7	0.496528879	0.530808855	0.416492601
203791_at	NM_005509.2	Hs.181042	0.053	-2.1677	DMXL1	0.645400966	0.960835018	1.226258193
204351_at	NM_005980.1	Hs.2962	0.0537	-2.1596	S100P	0.49193707	0.496153624	0.601000645
201170_s_at	NM_003670.1	Hs.171825	0.0546	-2.1507	BHLHB2	0.548460448	0.574865751	0.49210945
225046_at	BF667120	Hs.406650	0.0546	-2.1504	FLJ41510	0.523155822	0.568607967	0.662068658
225612_s_at	BE672260	Hs.136414	0.0573	-2.1225	B3GNT5	0.669623796	0.768179338	0.63246118
201473_at	NM_002229.1	Hs.400124	0.0573	-2.1224	JUNB	0.493732742	0.61851038	0.572322256
204582_s_at	NM_001648.1	Hs.171995	0.0601	-2.0949	KLK3	0.283429406	0.589742134	1.304985589
212789_at	AI796581	Hs.13421	0.0644	-2.0552	KIAA0056	0.608997484	0.939628975	1.410142531
203908_at	NM_003759.1	Hs.5462	0.0649	-2.0506	SLC4A4	0.513131934	1.481621069	2.537853202
201563_at	L29008.1	Hs.878	0.0654	-2.046	SORD	0.451194273	0.861192916	1.594819444
203574_at	NM_005384.1	Hs.79334	0.0695	-2.0109	NFIL3	0.565727477	0.577268422	0.650209608

Affymetrix ProbeSetName	Genbank	Unigene	Metastatic p-value	Metastatic t-statistic	Gene	Fold Change		
						Met-Nrml	G9 - Nrml	G6 - Nrml
206529_x_at	NM_000441.1	Hs.159275	0.0704	-2.0037	SLC26A4	0.551951321	0.631352534	0.66982304
211298_s_at	AF116645.1	Hs.184411	0.0708	2	ALB	4.038348409	1.02982235	1.072392767
222516_at	AA700485	Hs.298442	0.0677	2.0259	AP3M1	1.540043784	1.105426064	1.21683644
209160_at	AB018580.1	Hs.78183	0.0674	2.0289	AKR1C3	1.499988089	1.148809647	0.95052273
211110_s_at	AF162704.1	Hs.99915	0.0668	2.0338	AR	1.963334407	1.317125468	1.5340528
200598_s_at	AI582238	Hs.82689	0.0653	2.0467	TRA1	1.52452446	1.27999211	1.989934304
201852_x_at	AI813758	Hs.119571	0.0632	2.0658	COL3A1	1.902896136	1.730098336	0.796575886
227235_at	AI758408	Hs.22247	0.0619	2.0778	FLJ42250	1.576454945	1.289772714	1.496714465
229530_at	BF002625	Hs.29088	0.0617	2.0801	IMAGE:3315604	1.65327194	1.327584952	1.629400268
226884_at	N71874	Hs.126085	0.0595	2.1008	LRRN1	1.548535045	1.363318876	1.312256682
201008_s_at	NM_006472.1	Hs.179526	0.0575	2.1211	TXNIP	1.799826636	1.161864435	1.552769217
226726_at	W63676	Hs.356547	0.0544	2.1531	LOC129642	1.703434777	1.376392585	1.615871928
223423_at	BC000181.2	Hs.97101	0.054	2.1563	GPCR1	1.764712506	1.80971944	2.088695561
217733_s_at	NM_021103.1	Hs.76293	0.0503	2.1978	TMSB10	1.503806522	1.109655595	1.077926843
216379_x_at	AK000168.1	Hs.375108	0.0499	2.2026	FLJ20161	1.825688217	1.303355294	1.586083962
213812_s_at	AK024748.1	Hs.108708	0.0497	2.2039	CAMKK2	1.647330039	1.856918875	2.401956042
211161_s_at	AF130082.1	Hs.327412	0.0462	2.2467	FLC1492	1.848041612	1.554130932	0.94132736
220161_s_at	NM_019114.1	Hs.267997	0.0455	2.2553	EPB41L4B	1.512813189	1.488934601	1.573558969
225499_at	AW296194	Hs.17235	0.0439	2.2758	FLJ22541	1.620548305	1.466725395	1.475166509
227492_at	AI829721	Hs.171952	0.0427	2.2904	OCLN	1.541582175	1.377461428	1.232178281
218350_s_at	NM_015895.1	Hs.234896	0.0412	2.3115	GMNN	1.541471697	1.008334353	0.849756992
209613_s_at	M21692.1	Hs.4	0.0408	2.3166	ADH1B	2.004916435	0.962435512	0.837725721
209374_s_at	BC001872.1	Hs.153261	0.0393	2.3381	IGHM	1.816654151	1.305366845	1.032416003
226226_at	AI282982	Hs.283552	0.0359	2.3898	LOC120224	1.756061279	1.200620676	1.260631471
206351_s_at	NM_002617.1	Hs.247220	0.0347	2.4093	PEX10	1.622699512	1.27142138	1.489345755
211074_at	AF000381.1	Hs.73789	0.0326	2.4444	Folate binding protein	1.578683325	1.381413609	1.789411263
202427_s_at	NM_015415.1	Hs.76285	0.0323	2.4497	DKFZP564B167	1.670183347	1.351905473	2.246923836
201720_s_at	AI589086	Hs.79356	0.032	2.4552	LAPTM5	1.69885847	1.061164515	0.966340129
227197_at	AI989530	Hs.240845	0.0316	2.4606	DKFZP434D146	1.659535166	1.978903297	2.278268404
221942_s_at	AI719730	Hs.75295	0.0313	2.4669	GUCY1A3	1.844715047	1.448858579	2.085521221
233950_at	AK000873.1	Hs.151301	0.031	2.473	CADPS	1.546427503	1.085472457	0.984688555
217736_s_at	NM_014413.2	Hs.258730	0.0303	2.4847	HRI	1.536515183	1.604502316	1.817901191
208808_s_at	BC000903.1	Hs.80684	0.0295	2.501	HMGB2	1.675010385	1.162704083	0.924389164
204319_s_at	NM_002925.2	Hs.82280	0.0294	2.5022	RGS10	1.541898982	1.309324255	1.795358401
203215_s_at	AA877789	Hs.22564	0.0291	2.5082	MYO6	1.633958411	1.606283869	1.861691317

Affymetrix ProbeSetName	Genbank	Unigene	Metastatic p-value	Metastatic t-statistic	Gene	Fold Change		
						Met-Nml	G9 - Nml	G6 - Nml
202854_at	NM_000194.1	Hs.82314	0.0289	2.5108	HPRT1	1.529834801	1.179426162	1.174940245
202310_s_at	NM_000088.1	Hs.172928	0.0287	2.5162	COL1A1	2.033537613	1.914940515	0.772389958
206214_at	NM_005084.1	Hs.93304	0.0285	2.519	PLA2G7	1.605980146	1.707204536	1.777048436
217871_s_at	NM_002415.1	Hs.73798	0.0283	2.5237	MIF	1.769625594	1.343349079	1.596049197
209424_s_at	NM_014324.1	Hs.128749	0.0281	2.5272	AMACR	2.116938837	2.324343802	5.066327548
217848_s_at	NM_021129.1	Hs.184011	0.0255	2.5829	PP	1.711672524	1.14995071	1.246624657
220199_s_at	NM_022831.1	Hs.107637	0.0238	2.6218	FLJ12806	2.391285989	1.145492807	1.121762377
208905_at	BC005299.1	Hs.169248	0.022	2.6644	CYCS	1.570755038	1.345901439	1.3984069
224840_at	AL122066.1	Hs.7557	0.0218	2.6687	FKBP5	1.48846771	1.036856486	1.850099599
229152_at	AI718421	Hs.320147	0.0216	2.6754	C4ORF7	2.322871439	0.998617569	0.971594162
203431_s_at	NM_014715.1	Hs.111138	0.0216	2.6762	RICS	1.52225145	1.312998997	1.230108289
205943_at	NM_005651.1	Hs.183671	0.0209	2.6944	TDO2	1.760600293	1.50100665	1.188986943
201422_at	NM_006332.1	Hs.14623	0.0206	2.7003	IFI30	1.552309296	1.136298126	0.932541939
218559_s_at	NM_005461.1	Hs.169487	0.0205	2.704	MAFB	1.565093687	1.168516107	1.174192575
226880_at	AL035851	Hs.118064	0.0198	2.7228	NUCKS	1.600299748	1.366839531	1.39888628
209875_s_at	M83248.1	Hs.313	0.0196	2.729	SPP1	1.778246021	1.51644862	1.275916329
226039_at	AW008441	Hs.24210	0.0187	2.7549	MGAT4A	1.627101772	1.219058919	1.187042252
225647_s_at	AI246687	Hs.10029	0.0185	2.7623	CTSC	1.501738811	1.165441402	1.098532931
224665_at	AK023981.1	Hs.178485	0.0176	2.7906	LOC119504	1.530272787	0.998417546	1.075123958
241926_s_at	AA296657	Hs.45514	0.0174	2.7956	ERG	1.914432841	1.28776349	1.496429254
201288_at	NM_001175.1	Hs.83666	0.0174	2.7963	ARHGDIB	1.83262893	1.014920395	1.014793823
229724_at	AI693153	Hs.1440	0.0171	2.8068	GABRB3	1.616657166	1.451776055	1.846212704
200644_at	NM_023009.1	Hs.75061	0.0163	2.8315	MLP	1.960047156	1.934633141	2.382304727
200665_s_at	NM_003118.1	Hs.111779	0.0158	2.8486	SPARC	1.839336794	1.422425643	0.906449465
224833_at	BE218980	Hs.18063	0.0156	2.8564	ETS1	1.769713096	1.01329137	0.985362417
204416_x_at	NM_001645.2	Hs.268571	0.015	2.8784	APOC1	2.659455722	1.314190401	1.206631876
218025_s_at	NM_006117.1	Hs.15250	0.0148	2.8861	PECI	1.556592348	1.317497889	1.73958772
200771_at	NM_002293.2	Hs.214982	0.0138	2.9251	LAMC1	1.551677343	1.021886887	0.909481221
217294_s_at	U88968.1	Hs.381397	0.0134	2.9417	ENO1	1.709198983	1.094746038	1.239077599
227405_s_at	AW340311	Hs.302634	0.0131	2.9538	FZD8	1.554378677	1.078120743	1.146047942
203910_at	NM_004815.1	Hs.70983	0.0129	2.965	PARG1	1.566658602	1.091725294	1.196943379
209781_s_at	AF069681.1	Hs.13565	0.0127	2.9699	KHDRBS3	1.720661696	1.119899822	1.079578584
200971_s_at	NM_014445.1	Hs.76698	0.0127	2.9726	SERP1	1.559636173	1.331160738	1.628062522
226801_s_at	W72220	Hs.107637	0.0123	2.9916	FLJ12806	2.393236703	1.243562888	1.140090384
211634_x_at	M24669.1	Hs.153261	0.0112	3.0444	IGHG1	2.59388633	1.360479452	1.073739062

Affymetrix ProbeSetName	Genbank	Unigene	Metastatic p-value	Metastatic t-statistic	Gene	Fold Change		
						Met-Nrml	G9 - Nrml	G6 - Nrml
207543_s_at	NM_000917.1	Hs.76768	0.0109	3.0555	P4HA1	1.733925706	1.252700489	1.186234466
210108_at	BE550599	Hs.399966	0.0109	3.0595	CACNA1D	1.489860167	1.384488076	1.495170472
203932_at	NM_002118.1	Hs.1162	0.0104	3.0864	HLA-DMB	1.524664331	1.189013209	1.06592707
203915_at	NM_002416.1	Hs.77367	0.0102	3.0926	CXCL9	1.909087593	1.2391476	1.074101762
221011_s_at	NM_030915.1	Hs.57209	0.0096	3.1259	LBH	1.81373734	1.470327604	1.270395433
200016_x_at	NM_002136.1	Hs.376844	0.0096	3.1299	HNRPA1	1.463719776	1.22408099	1.215486347
213187_x_at	BG538564	Hs.433669	0.0093	3.1451	FTL	1.664543605	1.167743171	1.128725875
206858_s_at	NM_004503.1	Hs.820	0.0093	3.1466	HOXC6	1.855396742	1.814474567	2.200409215
208308_s_at	NM_000175.1	Hs.406458	0.0091	3.1586	GPI	1.719772684	1.349627658	1.566825826
225155_at	BG339050	Hs.292457	0.0088	3.1758	LOC389414	1.699552974	1.495191613	1.42639293
200910_at	NM_005998.1	Hs.1708	0.0083	3.21	CCT3	1.636454945	1.407382031	1.738311083
201417_at	NM_003107.1	Hs.351928	0.008	3.2293	SOX4	1.970734373	1.650462431	1.909514117
200967_at	NM_000942.1	Hs.394389	0.0078	3.2452	PPIB	1.662514576	1.1363543	2.158290879
201947_s_at	NM_006431.1	Hs.432970	0.0078	3.2475	CCT2	1.542573507	1.444834092	1.532058132
208638_at	BE910010	Hs.372429	0.0077	3.2521	ATP6V1C2	1.583571942	1.051678053	1.649215708
213088_s_at	BF240590	Hs.44131	0.0077	3.2524	DNAJC9	1.522969245	1.19041669	1.101924249
201892_s_at	NM_000884.1	Hs.75432	0.0075	3.2688	IMPDH2	1.545438098	1.476483085	1.73248107
200921_s_at	NM_001731.1	Hs.77054	0.0069	3.3146	BTG1	1.737055883	1.190188086	1.085456613
208650_s_at	BG327863	Hs.375108	0.0067	3.3288	CD24	1.829886814	1.355111901	1.591094884
233955_x_at	AK001782.1	Hs.15093	0.0067	3.3325	HSPC195	1.532399783	1.179795978	1.338839462
210338_s_at	AB034951.1	Hs.180414	0.0066	3.3376	HSPA8	1.68010557	1.41400935	1.538594921
229742_at	AA420989	Hs.97896	0.0065	3.3477	LOC145853	1.576219764	1.281197519	1.630748937
216207_x_at	AW408194	Hs.390427	0.0063	3.3683	IGKC	2.280006856	1.312304195	0.97191288
200052_s_at	NM_004515.1	Hs.75117	0.0062	3.3732	ILF2	1.500432046	1.179963924	1.395549103
200751_s_at	BE898861	Hs.406125	0.0061	3.3834	HNRPC	1.534667928	1.184841638	1.366841459
205133_s_at	NM_002157.1	Hs.1197	0.006	3.3941	HSPE1	1.563125779	1.432509648	1.587948037
202345_s_at	NM_001444.1	Hs.153179	0.0059	3.4071	FABP5	1.540717022	1.936910892	2.933164929
224997_x_at	AL575306	Hs.352114	0.0057	3.4183	LOC283120	1.850665142	1.121867318	1.03987769
226243_at	BF590958	Hs.293943	0.0052	3.4762	LOC391356	1.594266731	1.313820503	1.983449106
226711_at	BF590117	Hs.106131	0.005	3.4963	HTLF	1.605953506	1.113911789	1.041441881
222976_s_at	BC000771.1	Hs.85844	0.0049	3.508	TPM3	1.595051354	1.196763387	1.15890854
225655_at	AK025578.1	Hs.108106	0.0048	3.5199	UHRF1	1.633324349	1.262569313	1.076492985
201730_s_at	BF110993	Hs.169750	0.0046	3.5406	TPR	1.65067228	1.276077237	1.489979997
209301_at	M36532.1	Hs.155097	0.0045	3.553	CA2	1.775302858	1.022589313	1.018671643
217989_at	NM_016245.1	Hs.12150	0.0043	3.578	RETSDR2	1.723038343	1.105299082	1.319059719

Affymetrix ProbeSetName	Genbank	Unigene	Metastatic p-value	Metastatic t-statistic	Gene	Fold Change Met-Nml	G9 - Nml	G6 - Nml
212884_x_at	AI358867	Hs.169401	0.0043	3.5876	APOC4	2.131295433	1.351949253	1.23086617
202016_at	NM_002402.1	Hs.79284	0.0041	3.6079	MEST	1.529459472	1.310502398	1.081141622
223034_s_at	BC000152.2	Hs.355906	0.0041	3.6103	NICE-3	1.66226553	1.326721145	1.506773141
229429_x_at	AA863228	Hs.379811	0.0041	3.616	IMAGE:6191689	1.515106064	1.321222321	1.214669373
200003_s_at	NM_000991.1	Hs.356371	0.0037	3.6632	RPL28	1.550101477	1.355858477	1.452357975
213366_x_at	AV711183	Hs.155433	0.0036	3.6807	ATP5C1	1.529032497	1.119093162	1.331117036
225340_s_at	BG107845	Hs.278672	0.0036	3.6813	M11S1	1.582161146	1.287025159	1.498201492
200738_s_at	NM_000291.1	Hs.78771	0.0036	3.6839	PGK1	1.683510425	1.072151437	1.244584776
211935_at	D31885.1	Hs.75249	0.0035	3.7007	ARL6IP	1.586948602	1.45583784	1.354948119
230875_s_at	AW068936	Hs.29189	0.0035	3.7026	ATP11A	1.893995893	1.28867867	1.284361224
211798_x_at	AB001733.1	Hs.102950	0.0032	3.7431	IGLJ3	2.253481227	1.190254197	0.949978045
201258_at	NM_001020.1	Hs.397609	0.0032	3.7555	RPS16	1.529474743	1.257275471	1.240593812
200046_at	NM_001344.1	Hs.82880	0.0031	3.7691	DAD1	1.503927044	1.23704027	1.45535289
200023_s_at	NM_003754.1	Hs.7811	0.0031	3.7759	EIF3S5	1.492677918	1.053270057	1.303541027
200806_s_at	BE256479	Hs.79037	0.003	3.7832	HSPD1	1.963190492	1.71958284	1.754752854
201268_at	NM_002512.1	Hs.433416	0.003	3.7882	NME2	1.52341029	1.365066589	1.56867628
224599_at	BF570193	Hs.4867	0.003	3.7948	MGAT4B	1.622431221	1.358611937	1.359348866
200608_s_at	NM_006265.1	Hs.81848	0.0028	3.8326	RAD21	1.60409789	1.30816732	1.284445316
213872_at	BE465032	Hs.7779	0.0028	3.8362	C6ORF62	1.646199498	1.17809584	1.200055245
218188_s_at	NM_012458.1	Hs.23410	0.0027	3.8535	MKNK2	1.503773313	1.349464531	1.566222625
204714_s_at	NM_000130.2	Hs.30054	0.0026	3.8747	F5	2.165592205	1.679183224	1.676626265
200077_s_at	D87914.1	Hs.281960	0.0025	3.8866	OAZ1	1.524134063	1.262277281	1.230514687
213864_s_at	AI985751	Hs.302649	0.0025	3.8979	NAP1L1	1.67220728	1.394334759	1.301162479
201577_at	NM_000269.1	Hs.118638	0.0024	3.9233	NME1	1.762629579	1.473601738	1.768856036
212828_at	AL157424.1	Hs.417119	0.0024	3.9288	SYNJ2	1.558960833	1.215873328	1.26061887
200074_s_at	U16738.1	Hs.406451	0.0022	3.9762	RPL14	1.554434561	1.307651132	1.627182376
202779_s_at	NM_014501.1	Hs.174070	0.0022	3.9798	E2-EPF	1.567646954	1.295809911	1.146938081
211765_x_at	BC005982.1	Hs.401787	0.0021	3.9977	PPIA	1.573983335	1.425560154	1.374534514
208864_s_at	AF313911.1	Hs.432922	0.0019	4.0434	TXN	1.787154285	1.626360765	1.669494713
225541_at	BE274422	Hs.380933	0.0019	4.0627	LOC200916	1.542963884	1.631682436	1.778268586
212282_at	L19183.1	Hs.199695	0.0019	4.0627	MAC30	1.753247965	1.348307061	1.511823697
210024_s_at	AB017644.1	Hs.4890	0.0018	4.0888	UBE2E3	1.636706653	1.501673356	1.582518098
201923_at	NM_006406.1	Hs.83383	0.0018	4.0895	PRDX4	2.092722507	1.503078231	2.357995857
212085_at	AA916851	Hs.397980	0.0018	4.0911	SLC25A6	1.904390097	1.32734083	1.618458407
204934_s_at	NM_002151.1	Hs.823	0.0018	4.1026	HPN	1.960192099	1.784641097	2.452778498

Affymetrix ProbeSetName	Genbank	Unigene	Metastatic p-value	Metastatic t-statistic	Gene	Fold Change Met-Nrml	G9 - Nrml	G6 - Nrml
227558_at	AI570531	Hs.5637	0.0017	4.1127	CBX4	1.50757404	1.452066169	1.692781886
203663_s_at	NM_004235.1	Hs.434076	0.0017	4.1185	COX5A	1.613062245	1.374743859	1.755673991
218226_s_at	NM_004547.2	Hs.227750	0.0016	4.1453	NDUFB4	1.742463447	1.359340208	1.586965718
200089_s_at	AI953886	Hs.286	0.0016	4.1592	RPL4	1.53268956	1.115222706	1.484095711
201091_s_at	BE748755	Hs.406384	0.0015	4.1926	CBX3	1.524136246	1.380672462	1.217491886
224779_s_at	AI193090	Hs.406548	0.0015	4.2067	FLJ22875	1.558101693	1.273161615	1.427956916
206052_s_at	NM_006527.1	Hs.75257	0.0015	4.2109	SLBP	1.521358079	1.252449739	1.271120912
200099_s_at	AL356115	---	0.0015	4.2143	RPS3A	1.520554944	1.143653686	1.248258538
203593_at	NM_012120.1	Hs.374340	0.0014	4.2363	CD2AP	1.602425228	1.242316644	1.5150563
223015_at	AF212241.1	Hs.332404	0.0014	4.2391	EIF2A	1.497306539	1.242359457	1.344582841
219065_s_at	NM_015955.1	Hs.20814	0.0013	4.268	CGI-27	1.507583206	1.328804481	1.277143137
226431_at	AK025007.1	Hs.283707	0.0013	4.2731	FLJ38771	1.598874153	1.399493212	1.627928293
205967_at	NM_003542.2	Hs.46423	0.0013	4.3018	HIST1H4C	1.555503253	1.087464227	1.116349924
212582_at	AB040884.1	Hs.109694	0.0012	4.311	OSBPL8	1.715379905	1.301229214	1.229883545
215785_s_at	AL161999.1	Hs.258503	0.0012	4.3179	CYFIP2	1.56203664	1.078404104	1.115902029
200005_at	NM_003753.1	Hs.55682	0.0012	4.3351	EIF3S7	1.486307905	1.092082639	1.35598979
201406_at	NM_021029.1	Hs.178391	0.0012	4.3469	RPL36AL	1.622586596	1.318939119	1.315227712
202589_at	NM_001071.1	Hs.29475	0.0011	4.3893	TYMS	1.767443638	1.222542727	1.002726592
200705_s_at	NM_001959.1	Hs.279859	0.0011	4.4036	EEF1B2	1.760982804	1.031697881	1.234933
203381_s_at	N33009	Hs.169401	0.001	4.4505	APOE	3.625071725	1.645068079	1.546251347
201909_at	NM_001008.1	Hs.180911	0.001	4.4516	RPS4Y	1.599654206	1.115641351	1.24634976
200651_at	NM_006098.1	Hs.5662	0.0009	4.4929	GNB2L1	1.588142549	1.229268774	1.528267857
204026_s_at	NM_007057.1	Hs.42650	0.0009	4.4937	ZWINT	1.59878202	1.294302945	1.152947206
211430_s_at	M87789.1	Hs.300697	0.0009	4.5085	IGHG3	6.771934405	1.802655294	1.254577557
222981_s_at	BC000896.1	Hs.236494	0.0008	4.5616	RAB10	1.529122674	1.169831372	1.184935217
204170_s_at	NM_001827.1	Hs.83758	0.0007	4.5462	CKS2	1.505806628	1.351484868	1.316478404
202233_s_at	NM_006004.1	Hs.73818	0.0006	4.7216	UQCRH	1.507080143	1.407548974	1.450326381
213941_x_at	AI970731	Hs.301547	0.0006	4.7385	RPS7	1.736561496	1.299553424	1.383761007
201931_at	NM_000126.1	Hs.169919	0.0006	4.7667	ETFA	1.518847136	1.235640895	1.484000826
200062_s_at	L05095.1	Hs.356255	0.0006	4.7681	RPL30	1.477700403	1.325310565	1.28346032
200024_at	NM_001009.1	Hs.356019	0.0004	4.9825	RPS5	1.543956946	1.237096382	1.41156327
212320_at	BC001002.1	Hs.179661	0.0004	5.0086	OK/SW-CL.56	1.549636979	1.09363087	1.144937515
221253_s_at	NM_030810.1	Hs.6101	0.0003	5.1364	TXNDC5	1.673690073	1.214164697	1.547677512
203213_at	AL524035	Hs.334562	0.0003	5.1385	CDC2	1.701034927	1.283019117	1.112690554
210027_s_at	M80261.1	Hs.73722	0.0003	5.1408	APEX1	1.569470289	1.267708436	1.408847905

Affymetrix ProbeSetName	Genbank	Unigene	Metastatic p-value	Metastatic t-statistic	Gene	Fold Change Met-Nml	G9 - Nml	G6 - Nml
200657_at	NM_001152.1	Hs.79172	0.0003	5.1983	SLC25A5	1.901741191	1.22604677	1.387961859
234000_s_at	AJ271091.1	Hs.260622	0.0003	5.2335	HSPC121	1.938344455	1.478824195	1.874867572
200022_at	NM_000979.1	Hs.405036	0.0003	5.2504	RPL18	1.498415215	1.119390181	1.333477902
212298_at	BE620457	Hs.69285	0.0003	5.256	NRP1	1.957544223	1.040349319	1.015201245
224841_x_at	BF316352	Hs.289721	0.0002	5.3063	LOC348531	1.857150338	1.760196863	1.784970922
203316_s_at	NM_003094.1	Hs.334612	0.0002	5.3428	SNRPE	1.806026674	1.369724754	1.387729965
214512_s_at	NM_006713.1	Hs.349506	0.0002	5.3545	PC4 (RNA pol II cofactor4)	1.532818871	1.168971448	1.141341586
200025_s_at	NM_000988.1	Hs.402678	0.0002	5.3774	RPL27	1.508452832	1.19030353	1.243386992
225681_at	AA584310	Hs.283713	0.0002	5.3796	CTHRC1	2.020161016	1.80816774	0.951729083
201292_at	NM_001067.1	Hs.156346	0.0002	5.3883	TOP2A	1.833549424	1.291691262	1.079088914
200029_at	NM_000981.1	Hs.252723	0.0002	5.4248	RPL19	1.521872043	1.194836581	1.312202279
219315_s_at	NM_024600.1	Hs.25549	0.0002	5.4645	FLJ20898	1.64775771	0.990110268	0.953024778
201202_at	NM_002592.1	Hs.78996	0.0002	5.5703	PCNA	1.669445435	1.205345044	1.17023514
213801_x_at	AW304232	Hs.406309	0.0002	5.6419	LAMR1	1.632088937	1.399421585	1.383008918
211762_s_at	BC005978.1	Hs.159557	0.0001	5.6456	KPNA2	1.755103495	1.29090564	1.0705669
211963_s_at	AL516350	Hs.82425	0.0001	5.6682	ARPC5	1.586387629	1.137184649	1.069876176
215157_x_at	AI734929	Hs.172182	0.0001	5.7526	PABPC1	1.6139613	1.411844073	1.486133943
221923_s_at	AA191576	Hs.355719	0.0001	5.7669	NPM1	1.511565517	1.347070601	1.495278592
209773_s_at	BC001886.1	Hs.75319	0.0001	5.8026	RRM2	1.648429002	1.136861988	1.084243831
210470_x_at	BC003129.1	Hs.172207	0.0001	5.8383	NONO	1.539777316	1.18853499	1.265461231
212433_x_at	AA630314	Hs.356360	0.0001	5.8503	RPS2	1.523462718	1.358219429	1.33114119
200002_at	NM_007209.1	Hs.182825	0.0001	5.976	RPL35	1.551069832	1.305374553	1.391704639
213175_s_at	AL049650	Hs.83753	0.0001	5.9948	SNRPB	1.576875717	1.135824457	1.139655055
200081_s_at	BE741754	Hs.380843	0	6.4154	RPS6	1.483436564	1.122173181	1.255583773
202503_s_at	NM_014736.1	Hs.81892	0	6.5147	KIAA0101	1.790877795	1.270030091	1.192391312
218039_at	NM_016359.1	Hs.279905	0	6.5894	ANKT	1.906301812	1.308144135	1.15463799
200823_x_at	NM_000992.1	Hs.350068	0	6.6909	RPL29	1.660135008	1.25782313	1.461476429
201592_at	NM_003756.1	Hs.58189	0	6.747	EIF3S3	1.624202671	1.284913932	1.214917882
200826_at	NM_004597.3	Hs.397090	0	8.4509	SNRPD2	1.668850891	1.095430311	1.237917587
224930_x_at	BE559788	Hs.99858	0	8.519	RPL7A	1.569935841	1.312951533	1.518175682
203554_x_at	NM_004219.2	Hs.252587	0	8.678	PTTG1	1.598399511	1.224970621	1.036680081

TABLE 3. Significance of the genes validated by Taqman real time PCR. Kruskal-Wallis Test was done to compare the medians between the groups. All seven validated down-regulated genes (PRIMA1, TU3A, KIAA1210, FLJ14084; SVIL, SORBS1 and C21orf63) are significantly decreased in Metastatic, Gleason 9 and Gleason 6 grades compared to benign tissues. The increase in the expression of genes (e.g., MAL2, MLP, SOX4 and FABP5) with 4-way null hypothesis and the 2-way null hypothesis of normal vs Gleason 6 tumors was significant. Two way null hypothesis of normal vs Metastatic was not significant for upregulated genes.

Kruskal-Wallis Test	P-values										
	Gene =	SORBS1	C21orf 63	SVIL	PRIMA1	FLJ14084	TU3A	KIAA1210	SOX4	MLP	FABP5	MAL2
Comparison		Down regulated							Up regulated			
	Nrml-Met-G6-G9	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001	0.0012	0.0032	0.0126	0.0358
	Met-G6-G9	0.0002	0.0021	0.0044	0.0110	0.0099	0.0098	0.0026	0.1096	0.4945	0.0316	0.6473
	Nrml-Met	0.0043	0.0043	0.0043	0.0043	0.0043	0.0043	0.0043	0.0918	0.2723	0.5101	0.0923
	Nrml-G6	0.0002	0.0002	0.0002	0.0004	0.0006	0.0002	0.0010	0.0061	0.0014	0.0097	0.0339
	Nrml-G9	0.0027	0.0001	0.0002	0.0003	0.0004	0.0011	0.0022	0.0002	0.0006	0.0998	0.0061
	Met-G6	0.0398	0.9580	0.0019	0.0027	0.0052	0.0037	0.0019	0.1021	0.6350	0.0268	0.4292
	Met-G9	0.0052	0.0114	0.0040	0.0145	0.0068	0.0088	0.0017	0.1898	0.5409	0.0734	0.8614
	G6-G9	0.0007	0.0021	0.8644	0.8452	0.8644	0.7884	0.9805	0.1497	0.2614	0.1243	0.4792

NOTES:
=>The 4-way null hypothesis is that the four medians are the same
=>The 3-way null hypothesis is that the three medians are the same
=>The 2-way null hypotheses are that he pair-wise medians are the same
=>Genes were sorted by the 4-way p-value